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VOL. 10 æ 1958 æ NO. 1

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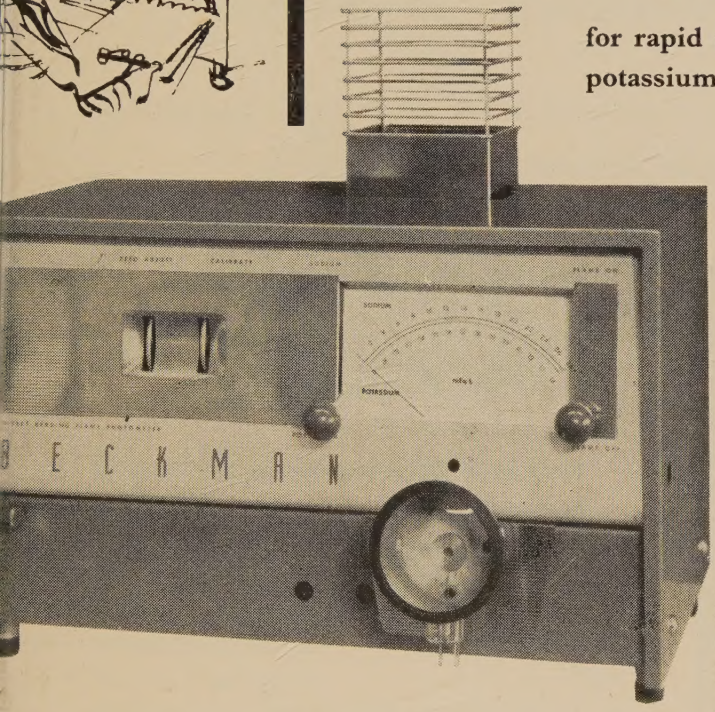

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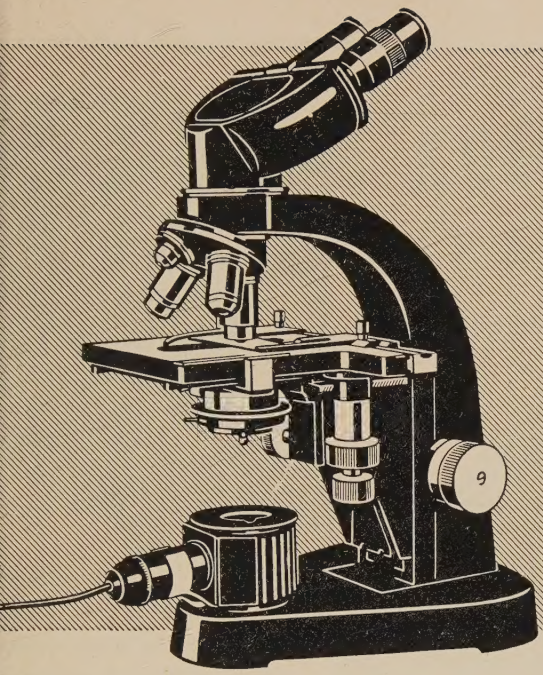

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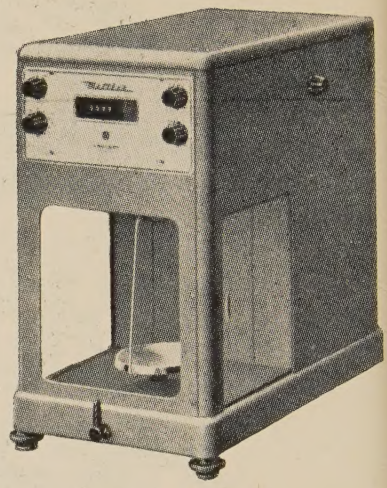

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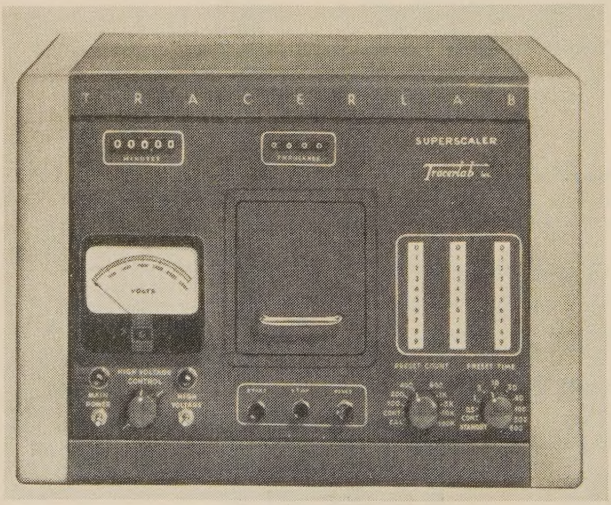

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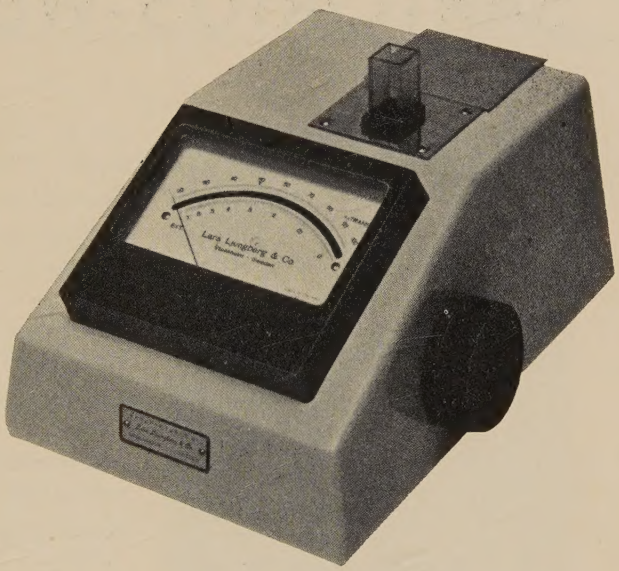

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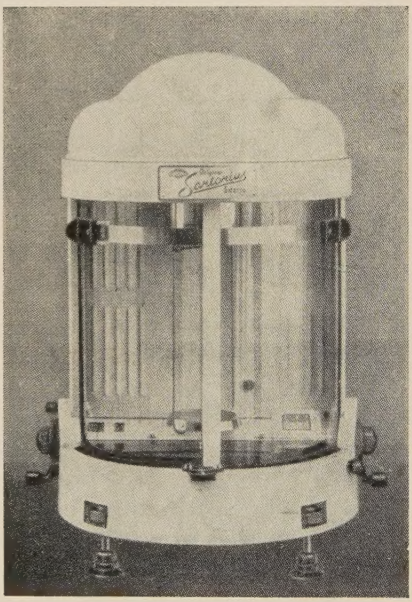

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Vol. 10 · 1958 · No. 1

av. J. Clin. & Lab. Investigation 1—4, 10, 1958.

OMPARISON BETWEEN MICRO-MODIFICATIONS OF ROTHROMBIN-PROCONVERTIN DETERMINATIONS AND OF THE PROTHROMBIN TIME METHOD [1]

By H. DYGGVE

From the Pediatric Department and the Department of Obstetrics A, Rigshospitalet, University Hospital, Copenhagen, Denmark

(Received for publication June 6, 1957)

theoretical and practical problems in- in determination of the prothrombin y of plasma and blood, especially in wborn, have been thoroughly discussed sen (1952).

the present investigation the two ls compared are Dyggve & Lund's cation of Owren's method (modified s, 1952) for the determination of the ied effect of prothrombin and procon- (the so-called PP-value) and Larsen m's modification (1941) of Quick's l prothrombin time method, a micro- l which has been used extensively in andinavian countries.

omparison of the PP-values obtained ie Owren-Aas technique and with our howed good agreement (Dyggve & Lund, 1954; Lund & Dyggve, 1954). Our method and the method of Larsen & Plum have been used with both capillary and venous blood without any significant difference in the results. Plum and Larsen consider the best way of indicating the result of the prothrombin time method is to give the time in seconds and simultaneously the time obtained in a normal adult with the same thromboplastin preparation. On the other hand, Lund, Geil, Söndergaard & Dam (1955) prefer to calculate the "Prothrombin Index" as the ratio between the prothrombin time of a normal individual and that of the patient. In this paper I prefer calculating the prothrombin time as prothrombin percentage using the graph of Larsen (1952, p. 80; cf. Plum, 1943).

It has been clearly shown, both in patients treated with dicumarol and in the newborn, that a decrease of prothrombin as well as

pported by a grant from the Danish State h Foundation.

proconvertin in plasma is a constant finding. Since both factors are influenced by vitamin K it seems desirable to compare the two methods. The advantage of micro-methods, especially in infants, is obvious.

MATERIAL AND METHODS

The method of Larsen & Plum (1941; see also Plum & Larsen, 1941 and 1942) was employed using frozen human brain thromboplastin (Dam & Glavind, 1938) and blood from a briskly bleeding heel puncture with mixture in their special pipette. With this method the normal time for adults is 18—19 seconds. The result was calculated as percentage of the normal adult value obtained the same day with the same thromboplastin preparation. The standard graph of Larsen (1952, p. 80) was made by comparison of the prothrombin times (Larsen & Plum method) with percentages obtained on the same blood using a modified Thordarson dilution method (cf. Plum, 1943). Larsen found correct results when a number of experiments, with dilution of normal blood with newborn blood with greatly prolonged prothrombin times, were plotted on his graph. There will be no appreciable difference with different preparations of thromboplastin, provided these are made according to Dam & Glavind.

The technique of Dyggve & Lund (1954) was used for the PP-method. The human brain thromboplastin and ox plasma were prepared according to Owren & Aas (1951). We use 0.1 ml capillary blood quickly mixed with the sodium citrate solution in a special pipette. After centrifugation and determination of the coagulation time of the plasma, the result is given in per cent of the normal value using specially prepared graphs.

Approximately one hundred newborn infants were used in this investigation. The actual number of newborns examined on the different days of life varied between 83 and 112. No vitamin K preparations were administered either to the mothers or the infants. The material includes no infants with birth weight under 2000 g. No case of hemorrhagic disease occurred during the period of investigation. Only results obtained with both methods on the same infant at the same intervals after birth are included. The determinations were made either by the author or by a specially trained technician under his close supervision.

RESULTS AND DISCUSSION

The results are presented in Fig. 1. It is seen that there is practically no difference between the distribution of the values obtained by the two methods. The percentages obtained by the PP-method tended to be a little lower than those found with the prothrombin time method. Especially the percentage of infants with PP-values below per cent of the normal adult values was considerably higher than the percentage of infants with such low values with the Larsen-Plum technique. This is not apparent from the figure. Fig. 1 shows that in general the results were quite similar. In the author's opinion this indicates that the method of Larsen & Plum is just as reliable as the other method during the postnatal period. Thus for practical purposes, the Larsen-Plum method probably measures quite accurately the combined decrease in prothrombin and proconvertin (factor VII) found in newborn infants.

The PP-method is somewhat more complicated than the Larsen-Plum method and it is necessary to store the ox plasma reagent at —20° C.

SUMMARY

One hundred newborn infants were examined daily during the first week of life both with a micro-modification of Owren's prothrombin-proconvertin method and with Larsen & Plum's prothrombin time method

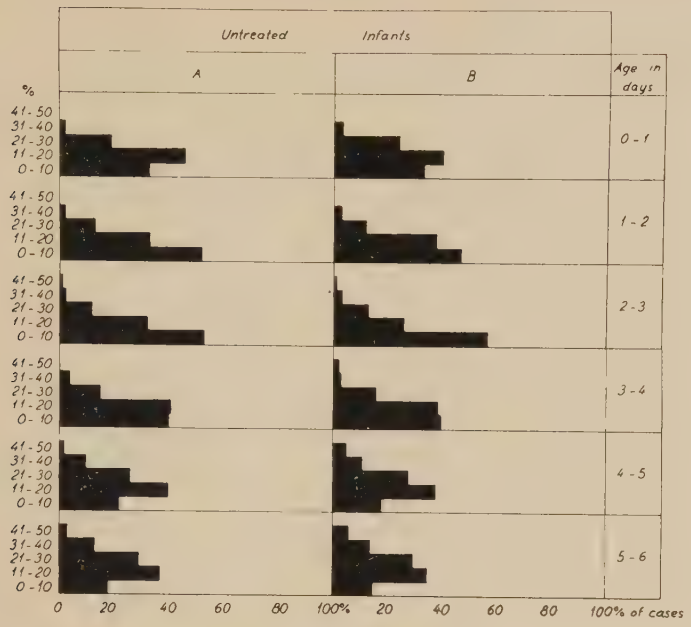

Fig. 1.

A: Distribution of prothrombin-proconvertin percentages (micro-method) and

B: Distribution of prothrombin time values (in per cent) of 100 newborn infants during the first six days of life.

Ordinate: A: Per cent of prothrombin-proconvertin (PP-value)

B: Per cent of "prothrombin activity".

Abscissa: Per cent of number of cases examined.

esults are given in per cent of the normal values. No vitamin K was administered either to the mothers or the infants. The only small differences were found between the results obtained by the two methods it is concluded that the Larsen-Plum method generally measures the combined deficiency in prothrombin and proconvertin ordinarily observed in the newborn.

REFERENCES

Aas, K.: Prokonvertin og konvertin. Diss. Oslo, 1952.

Dam, H. & Glavind, J.: The clotting power of human and mammalian blood in relation to vitamin K1. Acta med. Scandinav. *46*, 108, 1938.

van Creveld, S., Paulssen, M. M. P., Ens, J. C., Meil, C. A. M., Versteeg, P. & Versteegh, E. T. B.: Proconvertin content in the blood of

newborn full-term and premature infants. Études néo-natales *3*, 53, 1954.

Dyggve, H.: Undersøgelser over K-vitaminets betydning for blødninger hos nyfødte. Diss. Copenhagen, 1952.

Dyggve, H.: Prothrombin and proconvertin in the newborn and during the first year of life. Acta pædiatr. 1958. In press.

Dyggve, H. & Lund, E.: Micromethod for the quantitative determination of plasma prothrombin and proconvertin. Acta med. Scandinav. *150*, 207, 1954.

Larsen, Hj.: Svingningerne i prothrombinaktiviteten hos nyfødte. Diss. Copenhagen, 1952.

— & Plum, P.: Om prothrombinbestemmelser. Ugesk. f. læger *103*, 1273, 1941.

Lund, E. & Dyggve, H.: Control of dicumarol therapy by means of a micromethod for the quantitative determination of plasma prothrombin and proconvertin. Acta med. Scandinav. *150*, 271, 1954.

Lund, E., Geil, T., Søndergaard, E. & Dam, H.: Comparison of the prothrombin-proconvertin determinations (Owren-Aas) with "prothrombin-index" obtained with the Larsen-Plum prothrombin time method. Scandinav. J. Clin. & Lab. Investigation *7*, 69, 1955.

Owren, P. A. & Aas, K.: The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scandinav. J. Clin. & Lab. Investigation *3*, 201, 1951.

Plum, P.: Relation between prothrombin concentration and clotting time. Acta med. Scandinav. *115*, 41, 1943.

— & Larsen, Hj.: Über Prothrombinbestimmungen. Ztschr. f. klin. Med. *139*, 666, 194

— — Undersøgelser over prothrombinbestemmelser efter Quick's princip. Nord. Med. *16*, 340 1942.

Thordarson, O.: Undersøgelser over Prothrombin hos sunde og syge. Diss. Aarhus, 1941.

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DISTRIBUTION OF POTASSIUM EXCESS IN OLIGURIC PATIENTS

By STEFFEN GJØRUP and JØRN HESS THAYSEN[1]

The Metabolic Ward, Medical Dept. A, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark

(Received for publication September 14, 1957)

h present therapeutic procedures tis-
tabolism is inevitable in the oliguric
of acute renal failure. Among other
ts water and potassium are liberated
he catabolized tissue and must be eli-
d *via* extrarenal routes, if body com-
n is to be maintained within normal
Excess water is readily excreted with
sible perspiration" or *via* other extra-
routes, if care is taken that water in-
s kept sufficiently low to induce an
priate weight loss of the patient. The
of potassium to water excreted *via*
enal routes is, however, far lower than
io of potassium to water in catabolized
Consequently, a relative potassium
must inevitably develop in the oliguric
sm.

so far as therapeutic attempts effecti-
slow down or arrest potassium release
abolism have hitherto failed, the aim
tment must be to remove excess potas-
rom the oliguric organism. For prac-
urposes plasma potassium concentra-
used as a measure of potassium excess,
e effect of therapeutic procedures to
t potassium intoxication is assessed by
bility to reduce or normalize an eleva-

pported by a grant from "Reinh. W. Jorck
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ted concentration of potassium in the plasma. Extracellular potassium accumulation and total potassium excess are, however, not necessarily quantitatively related, for which reason therapy guided by plasma potassium concentration may not meet the theoretical ideal requirements outlined above. In view of the rapidity with which potassium generally reaccumulates in the extracellular fluid following *e. g.* dialytic removal of potassium, this question is of considerable practical importance and the purpose of the present study was therefore:

1) to assess to what extent extracellular potassium accumulation reflects total potassium excess in the oliguric patient, and

2) to ascertain whether variations in the total quantity of potassium removed during dialyses affect the rate of reaccumulation of potassium in the extracellular fluid in the period following dialytic treatment.

MATERIAL AND METHODS

The study was performed on 19 patients with oliguria due to acute renal failure. 15 had acute renal failure following shock and 4 other renal diseases (bilateral renal cortical necrosis, acute glomerulonephritis, myeloma kidney). 8 were males and 11 females, average age was 49 years with a range from 19 to 72 years and average duration of severe renal failure (*i. e.* 24-hour endogenous crea-

tinine clearance below 5 ml/min.) was 15 days with a range from 5 to 51 days. The patients were treated according to conventional conservative measures described elsewhere (Thaysen, Gjørup & Killmann, 1957) and subjected to balance studies as regards *inter al.* sodium and potassium, intake of nitrogen and calories, urea excretion and daily weight recordings. In 11 patients conservative treatment was supplemented with a total of 22 hemodialyses. The balance studies were performed in periods starting on admission and following dialytic treatment when administered; the periods terminated on the day of maximal serum urea concentration (respectively dialysis).

Extracellular potassium accumulation (K_{ec}) was calculated as:

$$K_{ec} = \Delta K \times ECV$$

where Δ K is the rise in plasma potassium concentration over the period examined and ECV extracellular fluid volume calculated as 15 per cent of body weight at the middle of the period (Levitt & Gaudino, 1950).

Potassium excess in the oliguric organism (K_{excess}) was calculated over the same period as K_{ec} by the formula:

$$K_{excess} = K_{balance} + K_{liberated}$$

where

$$K_{balance} = K_{intake\ (peroral\ +\ parenteral)} - K_{excreted\ (renal\ +\ extrarenal)}.$$

Usually $K_{balance}$ was negative since exogenous potassium supply was generally omitted.

Assuming that 3 meq. of potassium are liberated for each g of nitrogen catabolized (Darrow, 1950).

$$K_{liberated} = N_{catabolized\ x\ 3}$$

where

$N_{catabolized} = \Delta$ Serum urea N $\times$ TBW + Urea N excreted, since the nitrogen intake was zero or negligeable.

TBW was calculated as 60 and 55 per cent of body weight in the middle of the period for men and women respectively.

Hemodialyses were performed from radial artery to antecubital vein, using an *Alwall* dialyzer (Model 1953) (Alwall *et al.*, 1947—56) with a membrane pump between artery and apparatus to secure a blood flow of about 250 ml/min. The dialyzing surface is about 2 sq. m and the average "urea clearance" about 120 ml/min. As the volume of the dialyzing fluid (which is changed every 1—2 hours) is known, analysis of the fluid yields information about the quantity of substances removed during the procedure.

RESULTS

The relation between extracellular potassium accumulation (K_{ec}) and potassium excess (K_{excess}) of oliguric organisms was examined in 16 of the 19 patients, either non-dialyzed (7 cases) or in the period anteceeding their first dialysis (9 cases); the remaining 3 patients were dialyzed shortly after admission, for which reason balance studies prior to the first dialysis were not possible. The regression of K_{ec} on K_{excess} was 0.13 with a large standard deviation of 0.03, but significantly different from the ratio of 1.00 which should have been expected if all excess potassium were accumulated extracellularly (Fig. 1). The mean ratio of $\frac{K_{ec}}{K_{excess}}$ for all 16 patients was 0.22 ($\pm$ 0.11)

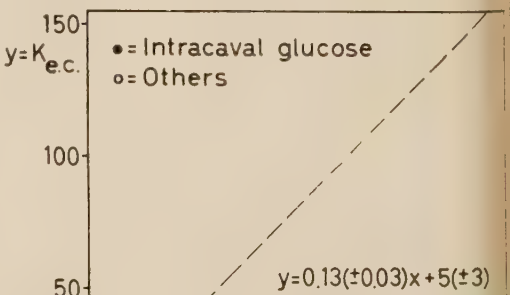

Fig. 1. The relation between extracellular potassium accumulation (K_{ec}) and total potassium excess (K_{excess}) in 16 balance periods of 16 patients.

was no significant difference of this etween 6 patients treated with intra- glucose infusion at the rate of about alories per day (mean ratio: 0.22 ± ind 10 patients receiving 100 calories ose or less per day (mean ratio: 0.11). The ratio $\frac{K_{oc}}{K_{excess}}$ was not antly affected by variations in the of acidosis as estimated by the stan- icarbonate concentration.

e findings demonstrate that only a 1 (in the present material about 1/5) ssium excess in the oliguric organism mulated in the extracellular fluid. val infusion of glucose at the rate of alories per day does not affect this of distribution, neither do moderate ns in the degree of acidosis.

The effect of acute potassium removal on plasma potassium concentration.

In 12 dialytic treatments on 8 patients dialysis was carried out against a potassium-free solution for 2—3 hours following which potassium was added to the dialyzing fluid in a concentration of 4.5 meq./liter. The measured total quantity of potassium removed in the 12 dialyses (mean 150 meq., range 82—260 meq.) was approximately equal to potassium excess in the oliguric organism on the day of dialytic treatment (mean of 8 dialyses on 6 patients in whom this calculation was possible: 97 meq. with a range from 8—153 meq.). The rate of reduction of plasma potassium concentration during dialysis is seen in Fig. 2 A.

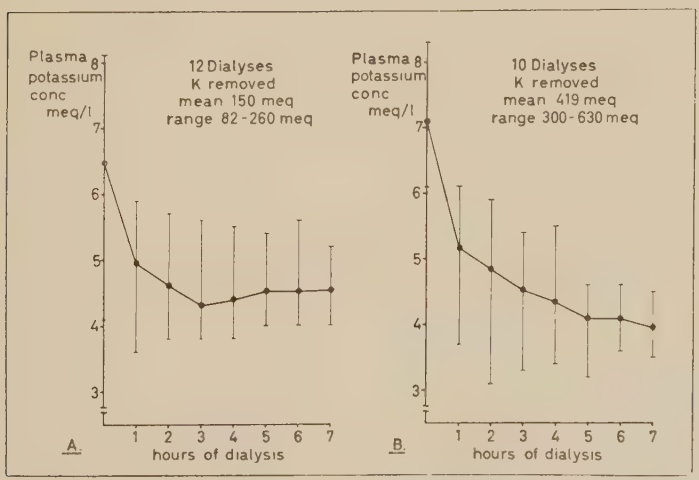

Fig. 2. The effect on plasma potassium concentration of varying degrees of potassium removal during dialysis. A. 12 dialysis with removal of an average of 150 meq. B. 10 dialyses with removal of an average of 419 meq.

In 10 dialytic treatments on 4 patients dialysis was carried out against a potassium-free solution throughout. The measured total quantity of potassium removed in the 10 dialyses (mean: 419 meq. with a range from 300 to 630 meq.) exceeded the potassium excess in the oliguric organism on the day of dialytic treatment (mean of 8 dialyses on 3 patients in whom calculation was possible 138 meq., range 31—276 meq.), indicating that definite depletion of body potassium was induced. Despite potassium depletion, plasma potassium concentration remained within normal range, although with a slightly falling tendency (Fig. 2 B).

With both procedures the amount of potassium removed from the extracellular compartment only represents a fraction of the total quantity of potassium removed, the greater amount being in all probability derived from intracellular pools. Since average rate of potassium removal by dialysis against potassium-free solution was 60 meq. per hour and since plasma potassium concentration was first normalized after 2 hours of dialysis against this solution it may, moreover, be concluded that removal of intracellular potassium occurs from the start of dialytic treatment and takes place even against an elevated concentration of potassium in the plasma. In the course of these 2 hours extracellular potassium was reduced by an average amount of 25 meq.; the ratio between average removal of extracellular potassium and average removal of total potassium (120 meq.) was, thus, about 1/5.

Effect of mere removal of potassium excess versus effect of potassium depletion on the rate of reaccumulation of potassium in the extracellular fluid. All of the 11 dialyzed patients survived their disease or lived sufficiently long to permit a study of this problem. Table I demonstrates that there was n

Table I. *Plasma potassium concentratio in the period following dialysis in two group of patients treated with varying degrees of potassium removal.*

Case No.	Dialysis No.	Potassium removed (meq)	Plasma potassium concentration (meq/l) on first 5 days following dialysis				
			1	2	3	4	5
Group A							
16	5	150	5.0	—	6.0	6.1	6.8
	6	100	6 6	7.4	7.2	5.6	6.9
18	7	150	4.4	4.3	5.1	—	5.7
30	20	260	4.4	5.2	6.0	6.4	7.1
	21	150	4.8	5.1	5.7	5.3	4.1
32	22	164	3.1	3.7	2.8	3.4	3.9
38	24	130	5.0	6.4	6.6	0	
39	25	82	6.3	6.2	*		
	27	—	6.2	5.5	8.0	7.5	0
44	30	110	5.2	6.4	6.7	8.1	—
	31	151	6.0	5.7	0		
46	32	205	5.1	5.5	5.5	6.5	7.2
		Mean 150	5.2	5.6	6.0		
Group B							
20	9	352	—	7.5	5.6	7.9	6.2
	10	350	0				
26	12	439	4.9	4.3	5.0	4.3	4.8
	14	300	3.9	3.7	4.4	4.2	4.5
	15	370	4.7	4.6	—	7.2	6.7
	18	356	4.8	4.5	5.2	5.5	6.6
27	16	467	5.3	6.1	7.3	*	
	17	300	5.2	5.4	7.1	7.3	*
	19	630	5.4	5.4	8.1	7.5	7.9
39	26	630	5.3	6.6	7.4	*	
		Mean 419	4.9	5.3	6.3		

0 = dead.
* = repeated dialysis.

e between the two dialytic procedu-
gards the rate of increase in the con-
n of potassium in the plasma in the
lowing dialysis. To exclude that this
difference between the two groups
to differences in K_{excess} per 24 hours
e ratio $\frac{K_{ec}}{K_{excess}}$, these two parameters
lculated for each group separately.
alues for K_{excess} per 24 hours in
and group B were 19 and 20 meq.
vely, or almost identical. Mean values
ratio $\frac{K_{ec}}{K_{excess}}$ in the two groups were
0.10) and 0.30 ($\pm$ 0.16), resepecti-
t significantly different by a T-test.
findings demonstrate that potassium
during dialysis offers no advantage
re removal of potassium excess with
to the rate of reaccumulation of po-
in the extracellular fluid in the period
g dialytic treatment.

MMARY AND CONCLUSIONS

een oliguric patients were studied
e purpose of investigating a) the
tion of potassium excess in the oli-
rganism and b) the effect of varying
of dialytic potassium removal.
lance studies showed that only about
ie calculated potassium excess in oli-
atients was accumulated extracellu-
he remaining 4/5 were stored else-
robably within the cells. This pattern
bution was found to be independent
agnitude of potassium excess within
of variation from 13 to 157 meq.
hen dialysis was carried out against
ium-free solution until normalization
of plasma potassium concentration, the quantity of potassium removed into the dialyzing fluid was approximately equal to the calculated potassium excess of the dialyzed patients. Only about 1/5 of potassium removed under these conditions was derived from extracellular sources. The remaining 4/5 were probably derived from the cells, and removal of cellular potassium took place from the start of dialytic treatment, even against an elevated concentration of potassium in the plasma.

3. The characteristic distribution of potassium excess between extra- and intracellular compartment was, thus, maintained during gradual accumulation of potassium in, as well as during rapid removal of potassium from, the oliguric patient. It corresponds roughly to the distribution of a single intravascular potassium load in dogs (Winkler & Smith, 1938) and in cats (Wiede, 1939).

4. When dialysis was continued against a potassium-free solution following normalization of plasma potassium concentration, removal of potassium into the dialyzing fluid continued with unaffected rate and exceeded the calculated potassium excess of the dialyzed patients. Despite the fact that definite depletion was induced, there was no further significant decrease in plasma potassium concentration, and it must be assumed that the bulk of removed potassium was derived from intracellular sources.

5. This finding on the effect of acute potassium depletion in man corresponds to the results on dialytic potassium depletion in dogs (Weller, Lown, Hoigne, Wyatt, Criscitiello, Merrill & Levine, 1955). Apparently a normal plasma potassium concentration is maintained considerably longer under con-

ditions of potassium depletion than under conditions of potassium excess.

6. Theoretically it might have been expected that patients treated with mere removal of potassium excess and patients in whom actual potassium depletion had been induced would react differently to catabolic potassium release in the period following dialytic treatment. However, in the present series there was no significant difference between the two groups with respect to the rate of reaccumulation of potassium in the extracellular fluid.

REFERENCES

Alwall, N. & co-workers: On the artificial kidney. Series in Acta Med. Scandinav. 1947—56.

Darrow, D. C.: Body fluids physiology. N England J. Med. *242,* 1014, 1950.

Levitt, M. F. & Gaudino, M.: Measurements body water compartments. Am. J. Med. *9,* 2 1950.

Thaysen, J. H., Gjørup, S. & Killmann, S—A Acute renal failure. Danish Med. Bull. *4,* 1957.

Weller, J. M., Lown, B., Hoigne, R. V., Wya N. F., Criscitiello, M., Merrill, J. P. & Levi S. A.: Removal of potassium from dogs. Circ lation. *11,* 44, 1955.

Wiede, W. S.: The distribution of potassium in cat after intravascular injection. J. Biol. Che *128,* 309, 1939.

Winkler, A. W. & Smith, P. K.: The appare volume of distribution of potassium inject intravenously. J. Biol. Chem. *124,* 589, 19

v. J. Clin. & Lab. Investigation 11—15, 10, 1958.

THE RADIOACTIVE IODINE EXCRETION TEST IN RENAL FAILURE

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(Received for publication September 20, 1957)

value of the radioactive iodine tech- n the diagnosis of thyroid disorders well established. In these procedures, e direct uptake of ^{131}I by the thyroid nd the excretion of ^{131}I in the urine en used as indicators. The reliability atter method, *i. e.* measurement of the tivity in the urine during the first 24 hours after administration of a dose has been proved (Skanse, 1949). The on method seems to be the method of in Finland, where goitre is endemic e thyroid glands of different subjects ry much in size and shape (Lamberg, erg & Kuhlbäck, 1956, and others). hyrotoxicosis, the bulk of the admi- d radioiodine is trapped by the thyroid and the urine excretion of ^{131}I is thus n earlier investigations, it has been that in thyrotoxicosis the 48 hour dine excretion is usually below 30 per f the dose administered. This figure e set against the background of the h goitre endemic, since in areas with ic goitre there is a tendency for iodine rapped even in euthyroidism. In hypo- lism, the 48 hour excretion is usually 0 per cent, and it is noted that on the day especially (24—48 hours) there ually high excretion values (Lamberg, Wahlberg, Kuhlbäck, Hernberg & Stenius, 1957).

Like every other excretion test, the ^{131}I test also depends on renal function. Free iodine, and hence also ^{131}I, is ultrafiltered in the glomeruli and is reabsorbed in the tubules — probably by a passive process — to about 50 or 70 per cent. In this respect, iodine has been compared with urea, and the ^{131}I clearance has been showed to be of the same order of magnitude as the urea clearance (Bricker & Hlad, 1955).

It is thus clear that the excretion of radioactive iodine must diminish if renal function is impaired. This fact has been stressed by Skanse (1949), who in ten patients with renal failure saw a more or less low excretion of radioactive iodine. The 24—48 hour excretion was proportionally higher than usual. Perry & Hughes (1952) observed a slightly decreased ^{131}I excretion in eight patients with renal failure. Castenfors, Ek & Porjé (1953) demonstrated a markedly diminished radioiodine excretion and clearance in four patients with severe nephritis (inulin clearance 3—7 ml/min.).

The present study of the ^{131}I excretion in renal failure has been carried out for the purpose of demonstrating the degree to which renal function must be impaired in order to

Table I. *The ^{131}I excretion test in control subjects.*

Case No.	Endogenous creatinine clearance ml/min.	Percentage ^{131}I excretion			Mean daily urine output ml
		0—24h	24—48h	0—48h	
1	145	31.3	1.9	33.2	1190
2	143	27.1	1.5	28.6	1710
3	125	66.5	0.9	67.4	2340
4	125	30.9	4.5	35.4	630
5	120	48.3	2.7	51.0	1450
6	106	25.0	0.5	25.5	1040
7	102	40.0	0.2	40.2	1320
8	102	24.4	0.4	24.6	910
9	99	28.3	1.3	29.6	2490
10	95	46.0	2.4	48.4	1120
11	90	52.4	5.7	58.1	1940
12	85	26.8	1.0	27.8	1790
13	85	46.5	0.9	47.4	2210
14	84	50.9	3.5	54.4	1600
15	65	26.1	4.6	30.7	930

disturb the radioiodine excretion, and ho the excretion pattern is influenced by re failure.

MATERIAL AND METHODS

The subjects investigated were 15 controls (wit out demonstrable thyroid or renal disease), 2 patients with renal failure (chronic nephropathy i steady state), 20 patients with thyrotoxicosis an 8 with hypothyroidism. The ^{131}I excretion test wa performed according to methods previously descri bed (Lamberg, Wahlberg & Kuhlbäck, 1956; Wah berg *et al.*, 1957). About 50 μC of ^{131}I was adm nistered by mouth, and the 0—24 and 24—48 hou urine was collected and its radioactivity determine with a scintillation counter. As an approxima indicator of renal function the endogenous creati ine clearance test was used. It was performed a cording to standard principles as described pre ously (Kuhlbäck, 1957). Two clearance periods o 2 hours each were used.

Table II. *The ^{131}I excretion test in renal failure.*

Case No.	Diagnosis	Endogenous creatinine clearance ml/min.	Percentage ^{131}I excretion			Mean daily urine output ml
			0—24 h	24—48 h	0—48 h	
16	Pyelonephritis	70	16.1	9.4	25.5	1080
17	Nephritis chr.	62	53.8	17.2	71.0	1120
18	Nephropathia chr.	56	27.0	3.0	30.0	1310
19	Pyelonephritis	45	26.0	12.5	38.5	1060
20	Nephropathia	40	23.5	7.2	30.7	1620
21	Nephropathia	39	27.5	6.8	34.3	1730
22	Nephritis chr.	29	26.7	7.1	33.8	2050
23	Nephritis chr.	29	7.8	0.0	7.8	2060
24	Praehydronephros	27	22.9	10.0	32.9	1970
25	Nephritis chr.	24	13.8	4.0	17.8	2280
26	Pyelonephritis	23	10.1	1.3	11.4	2330
27	Pyelonephritis chr.	22	11.9	5.0	16.9	1710
28	Nephritis chr.	20	23.8	13.0	36.8	2710
29	Nephrocirrhosis	17	23.4	7.2	30.6	2150
30	Pyelonephritis chr.	16	19.7	3.3	23.0	2035
31	Nephropathia chr.	11	11.7	1.3	13.0	4130
32	Pyelonephritis chr.	8	3.5	1.7	5.2	1380
33	Nephritis chr.	7	5.9	5.6	11.5	2660
34	Nephrosclerosis	3	5.9	4.4	10.3	1900
35	Pyelonephritis chr.	3	6.2	6.2	12.4	1900

e III. *The ^{131}I excretion test in thyrotoxicosis.*

Endogenous creatinine clearance ml/min.	Percentage ^{131}I excretion		
	0—24h	24—48h	0—48h
179	13.8	1.0	14.8
153	13.5	1.7	15.2
142	9.5	1.1	10.6
142	26.2	2.2	28.4
141	16.9	2.0	18.9
134	13.2	0.8	14.0
130	8.7	2.1	10.8
126	15.1	0.6	15.7
123	11.9	1.4	13.3
117	3.8	0.8	4.6
114	13.9	1.3	15.2
114	12.1	2.5	14.6
110	5.5	1.5	7.0
108	13.2	1.4	14.6
107	13.3	2.0	15.3
106	17.0	3.2	20.2
104	13.4	0.8	14.2
99	20.6	3.5	24.1
87	2.5	0.7	3.2
83	7.2	1.5	8.7

RESULTS

esults are compiled in Tables I—IV. ues for the ^{131}I excretion in normal as well as in thyrotoxicosis and roidism are in accordance with earlier from Finland (Lamberg *et al.*, 1957, rg *et al.*, 1957). It must be noted that the control subjects display an ex- which is below the normal 30 per is is due to the occurrence of an in- thyroid affinity for iodine in an en- oitre area. The low total excretion in xicosis and the high excretion during nd twenty-four hours in hypothyro- ere notable.

nal failure, the results were dissimi- he endogenous creatinine clearance ve 30 ml/min., the 0—48 hour ^{131}I excretion was within normal limits, but the 24—48 hour excretion was higher than normal. When the clearance values were 15—30 ml/min., the 0—48 hour radioiodine excretion was normal in some patients, in others decreased. The 24—48 hour value was sometimes increased. When the clearance values were below 15 ml/min., the 0—48 hour radioiodine value was always decreased, and the 24—48 hour value high in proportion to the 0—24 hour value. It is seen that radioiodine excretion was independent of diuresis.

The results are further elucidated by a diagram (Fig. 1).

DISCUSSION

The results show that renal failure may considerably disturb the ^{131}I excretion test. In mild renal failure, the high excretion during the second 24 hours may suggest hypothyroidism if the renal disease is not recognized. The clinical picture, with inertia, dry puffy skin and edema, may also sometimes be confusing. The situation is not clearer when one realizes that in hypo-

Table IV. *The ^{131}I excretion test in hypothyroidism.*

Case No.	Endogenous creatinine clearance ml/min.	Percentage ^{131}I excretion		
		0—24h	24—48h	0—48h
56	89	27.3	19.5	46.8
57	82	53.1	16.9	70.0
58	71	54.5	21.5	76.0
59	69	55.5	20.0	75.5
60	59	54.0	23.6	77.6
61	56	40.5	25.5	66.0
62	54	51.8	10.6	62.4
63	54	72.4	17.6	90.0

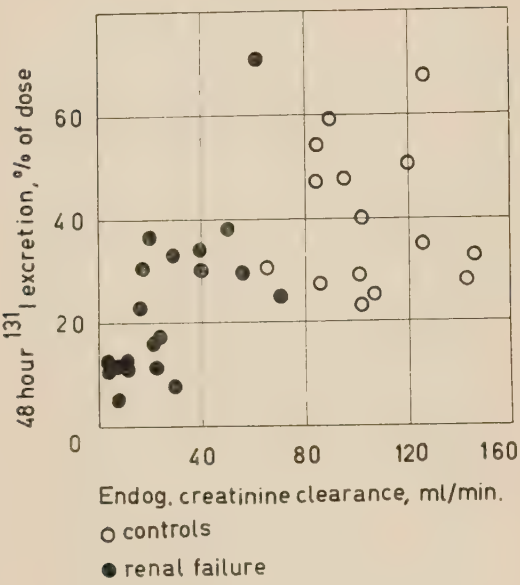

Fig. 1. The correlation between the 48 hour ^{131}I excretion and the endogenous creatinine clearance in renal failure and in controls.

thyroidism kidney function is somewhat impaired (Beaumont & Robertson, 1943; Corcoran & Page, 1947; Hlad & Bricker, 1954; Kuhlbäck, 1955; Yount & Little, 1955), and in thyrotoxicosis slightly increased (Aas & Blegen, 1949; Hlad & Bricker, 1954; Kuhlbäck, 1955). The 0—48 hour excretion, however, is usually higher in hypothyroidism than in renal failure. Usually the history and clinical and laboratory tests, of course, readily allow of a correct diagnosis.

In severe renal failure, the 48 hour excretion of ^{131}I is very low, in the same way as in thyrotoxicosis. In renal failure, however, the second day's excretion is proportionally high compared with the corresponding value in thyrotoxicosis, where it is low.

Direct measurement of the radioactivity over the thyroid gland may perhaps give better results than the excretion test in the cases, but it must be borne in mind that t delayed excretion through the kidneys pla a role here, too. The radioactive iodine p remains high for an unusually long time excretion through the kidneys is poor, a thus the thyroid may take up radioiod over a longer period than usual. The dir measurement of radioactivity over the th roid is not suitable where goitre is ende as is the case in Finland.

SUMMARY

The ^{131}I excretion test was studied patients with renal failure. If the endogeno creatinine clearance was above 30 ml/mi the 48 hour radioiodine excretion was fou to be normal, but the 24—48 hour value w higher than usual. When the clearance valu were 15—30 ml/min., low values of the hour excretion were sometimes seen, t 24—48 hour excretion was occasionally hig If the clearance was below 15 ml/min., t 48 hour excretion was low and the 24— hour value proportionally high.

It is pointed out that the ^{131}I excreti values in mild renal failure may sugg hypothyroidism, whereas the values in seve renal failure may be similar to those in thyr toxicosis.

REFERENCES

Aas, K. & Blegen, E.: The renal blood flow a the glomerular filtration rate in congest heart failure and some other clinical disord Scandinav. J. Clin. & Lab. Investigation *22*, 1949.

Beaumont, G. E. & Robertson, J. D.: Renal fu tion in myxedema. Brit. Med. J. *2*, 578, 19

Bricker, N. S. & Hlad, C. J. Jr.: Observations the mechanism of the renal clearance of I J. Clin. Investigation *34*, 1057, 1955.

s, H., Ek, J. & Porjé, I. G.: Urinutsönd- och clearance av J^{131} vid olika kliniska nd. Nord. Med. *50,* 1325, 1953.

A. C. & Page, I. H.: Specific renal is in hyperthyroidism and myxedema. in. Endocrinol. *7,* 801, 1947.

J. Jr. & Bricker, N. S.: Renal function I^{131} clearance in hyperthyroidism and dema. J. Clin. Endocrinol. & Metab. *14,* 1954.

, B.: Clearancebestämningar som njur- ionsprov. Finska läk. sällsk. handl. *98,* 955.

bleeding tendency in chronic renal failure. med. Scandinav. *157,* 173, 1957.

B.-A., Wahlberg, P. & Kuhlbäck, B.: xkretionen vid endemisk struma i Finland. . Med. *55,* 354, 1956.

Lamberg, B.-A., Wahlberg, P., Kuhlbäck, B., Hernberg, C. A. & Stenius, K.: An appraisal of the diagnostic significance of the radioactive iodine excretion test and the protein-bound iodine in the serum in an endemic goitre area. Acta med. Scandinav. *158,* 63, 1957.

Perry, W. F. & Hughes, J. F.: The urinary excretion and thyroid uptake of iodine in renal disease. J. Clin. Investigation *31,* 457, 1952.

Skanse, B.: Radioactive iodine in the diagnosis of thyroid disease. Acta med. Scandinav. suppl. *235,* 1949.

Wahlberg, P., Lamberg, B.-A., Kuhlbäck, B., Hernberg, C. A. & Stenius, K.: The excretion of radioactive iodine in an endemic goitre area. Acta med. Scandinav. *158,* 55, 1957.

Yount, E. & Little, J. M.: Renal clearance in patients with myxedema. J. Clin. Endocrinol. & Metab. *15,* 343, 1955.

Scandinav. J. Clin. & Lab. Investigation 16—25, 10, 1958.

THE EFFECT OF SUBSTANCE P ON INTESTINAL MOTILITY IN MAN

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(Received for publication September 27, 1957)

Several investigations have shown that substance P, a biologically active polypeptide which normally occurs in intestinal and brain tissue, has a powerful stimulating effect on smooth muscle, especially on isolated intestinal segments (Euler & Gaddum, 1931; Euler, 1936; Pernow, 1953) but also on the intestine in situ (Gernandt, 1940). It has been claimed that substance P constitutes a stimulus for the spontaneous motility in the intestinal wall (Euler, 1936). This view is *i. a.* supported by our knowledge about the distribution of substance P in the different segments and tissue layers of the intestinal wall, the highest concentration being found in muscularis mucosae and the external muscular layers of the duodenum and the ileum (Pernow, 1951; Douglas, Feldberg, Paton & Schachter, 1951). Gernandt (1940), studying the effect of substance P on the peristaltic reflex, found that if substance P was administered, the peristaltic reflex was obtained at a lower intraluminar pressure than that otherwise required.

Substance P is known to exert its contracting effect mainly by a direct stimulation of the smooth muscle fibres, which implies that its effects are not influenced by nicotine, cocaine, or more specific ganglionic blocking agents (Pernow, 1953). As has recently been shown by Blair & Clark (1956), isola segments of rabbit intestine react less m kedly to substance P after cooling or un anaerobic conditions, which might imply t there is a neurotropic component as well the stimulating action of substance P. Th are also observations which suggest that t content of substance P in the intestinal w would partly be related to the nervous stru ture, especially the ganglion cells. Th Ehrenpreis & Pernow (1953) showed th the content of substance P in the aganglion portion of the rectosigmoid in Hirschsprun disease is essentially lower than in the c responding segments under normal con tions or in adjacent neurohistologically no mal segments.

The effect of substance P on the circulati has a yet not been studied in detail. Euler Gaddum (1931), and others, have sho that after intravenous injection substance produces a rapidly transient fall in blo pressure owing to peripheral vasodilatati Holton & Holton (1952) also have noted vasodilatory effect after intra-arterial inj tion into the denervated isolated rabbit e

Up to the present no studies have be carried out on the effect of substance P man, mainly for the reason that the availa preparations of substance P have not be

ficiently high degree of purity to allow
udies to be undertaken. This investiga-
the first of a series of studies in man
s made chiefly to study the effect of
ce P on the intestinal motility.

METHODS

Preparation of substance P

material used for the preparation of sub-
P was small intestine from cows. The
n and subsequent purification were done
g to the method described earlier (Pernow,
onsisting of precipitation with ammonium
and adsorption on aluminium oxide
:

activity of the preparations was 600 to 800
r mg. The active fractions were poured
erile conditions into ampoules, each holding
ts, evaporated to dryness *in vacuo* in cold
ed at —20° C. Under these conditions the
remained unchanged for more than one
t each investigation the required amount
ance P was dissolved in physiologic saline
The activity was checked regularly after
eriment. In connection with the evaporation
ss a decrease in activity of 5 to 25 per cent
erved.

Method for studies on isolated human intestine

pieces of intestines were obtained at opera-
astro-enterostomies, segmental resections in
cancer *etc.)*. The gut was immediately after
placed in Tyrode's solution at 37° C.
of intestine, about one cm of the circum-
and 2.5 cm in length, was then suspended
dinary intestinal bath, containing Tyrode's
at 35° C. The capacity of the bath was
d the bath fluid was aerated with 6.5 per
O_2 in O_2.

are indebted to AB Astra for carrying out
step in this procedure.

Studies on human subjects

The authors carried out preliminary experiments on themselves, in which substance P was injected subcutaneously and intravenously. The rest of the material consisted of eight persons between 15 and 75 years of age, six of whom had recovered from duodenal ulcer, one, born 1880, with advanced cancer of the stomach, and one patient with a dynamic ileus. The latter was a 48 year old woman in whom the clinical and roentgenologic picture was that of paralytic ileus. The subsequent investigation showed advanced terminal ileitis with partial mechanical obstruction of long standing, which had given rise to the paralytic condition.

The subjects had fasted for at least five hours before the investigation. They were given a barium meal, and after about ¾ hour the jejunum or a proximal loop of the ileum were filled with the contrast medium. The peristaltic movements in this region were then studied by repeated brief fluoroscopic observations and by cineradiographic recording. Physiologic saline solution was then given for 20 minutes by intravenous infusion, followed by infusion for 20 minutes of substance P in a total amount of 600 to 1000 units. During the infusions the peristaltic movements were studied every five minutes by screening and filming. After complete infusion the motility was checked for a further 45 minutes.

For radiographic recording on cine film we used a cinecamera type Arriflex (35 mm film) connected to a Philip's image intensifier. The image intensifier and its application in medical practice have earlier been discussed *i. a.* by Janker (1956) and Penning (1956). The camera speed used was constantly 16 frames per second. To obtain constant exposure of the film during the whole examinations, independently of the varying object thickness, amount of contrast media *etc.*, a millivoltmeter was connected to the intensifier. By this device the amount of light emanating from the intensifier could be evaluated directly, and the X-ray intensity be adjusted accordingly. The recordings were as a rule made in consecutive periods of 10 or 15 seconds. Screen-examinations were in the mean-time made in short periods at low X-ray intensity. The X-ray dose was reduced by using additional filters, for instance a thin layer of copper.

Unoperative recording of the blood pressure and the pulse rate was made continuously during the course of infusion.

RESULTS

Effect on isolated human intestine

The effect of substance P was studied on isolated segments of human duodenum, jejunum and ileum. Definite effects were obtained by very small amounts of substance P in all segments, jejunum and ileum, however, being much more sensitive than duodenum. In order to get the same degree of shortening about 4—5 times more substance P was needed for the duodenal segments than for the jejunum or ileum. In the ileum preparations 0.3 units per ml bath fluid produced contractions of about twice the spontaneous amplitude.

The action of substance P on the jejunum and ileum preparations consisted in an initial relaxation rapidly followed by a gradual increase in tone. The spontaneous pendulum movements increased in amplitude, remained unchanged, or even ceased entirely. Small concentrations of substance P usually produced an increase in the spontaneous movements only, while larger amounts gave rise to an increased tone. The initial relaxation was not seen in the duodenal segments. The slow-reacting type of contraction, typical for substance P on all smooth muscle organs, is quite distinguishable from the rapid response, produced for instance by acetylcholine (Fig. 1).

Observations on human subjects

Subcutaneous injection: For the purpose of testing the tolerance to the preparation some preliminary experiments were made,

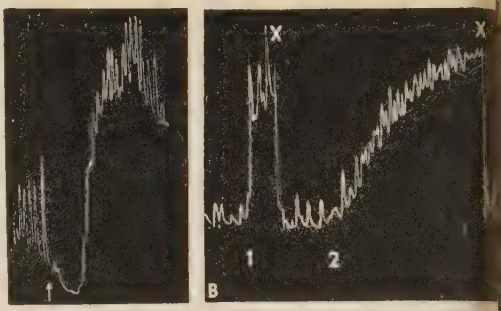

Fig. 1. Effect of substance P and acetylcholine the isolated human ileum (A) and duodenum (B Bath volume 15 ml. A: Effect of 10 units subst. P. B: 1 = 3 µg acetylcholine. 2 = 40 un of subst. P. At X changing of the bath fluid.

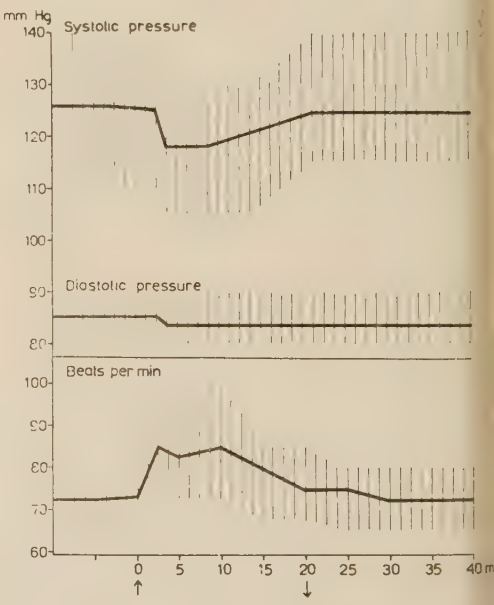

Fig. 2. Effect of substance P infusion on the blo pressure and pulse rate. 600 to 800 units were giv in 20 minutes infusion, the start and the end in cated by arrows. Full lines represent the me values for the whole group and vertical lines the individual scatter.

ing initially subcutaneous injections to 300 units. Within ½ minute a sensation occurred at the site of in- which lasted for a few minutes and sappeared completely. Besides a slight there was no local reaction. After 3 nutes the subjects experienced a feel- warmth in the face and there was a flushing of the face and the ears. lse-rate increased slightly, by a maxi- f 10 beats per minute. No effect was on either the systolic or the diastolic ressure. After 10 minutes the pulse- s the same as initially. Manifestations ased intestinal motility appeared after 0 minutes, consisting of borborygmia lic. The symptoms were reproduced ther subcutaneous injection of sub- P after four days. The local reaction the same order of magnitude as that e first injection.

avenous infusion: In connection with entgen-cinematographic studies sub- P was infused intravenously during 20 s in an amount varying between 600 00 units. The infusion started with of about 10 units per minute. Approxi- 1 minute after the beginning of in- marked flushing of the face and the vas seen. The subject experienced a of warmth in the face and marked ons at the temples. These symptoms only for a few minutes, even at an un- d rate of infusion. The flushes also eared within a few minutes. The rate usion could thereafter be increased ld without any further signs of flush jective symptoms. During the initial there was an average rise in the pulse- f 10 to 15 beats per minute, with a maximum of 20 beats per minute (Fig. 2). The blood pressure was unchanged in all cases (Fig. 2).

Within 5 minutes an increase in the intestinal motility was observed in all cases. This change was demonstrable for both the segmental and the peristaltic movements. In the fluoroscopic picture local deep constrictions of the intestinal lumen predominated, which indicated powerful contractions of the circular muscles. These constrictions shifted continuously both in the oral and the anal direction. In some areas the intestinal lumen diminished markedly, which suggested a general rise in the tone of the intestinal wall. Now and then powerful prolonged peristaltic waves were seen, which forced the content of the intestine in the direction of the colon (Fig. 3). Bowel sounds were heard at the same time, and in a few cases the subjects complained of attacks of colicky pain. The increased motility was fully observable during the whole period of infusion, and, on an average, for 10 to 20 minutes after the end of the infusion.

In the case of intestinal paralysis the gut was initially enormously distended and showed no types of movements during an observation time of one hour before and during saline infusion. The patient was thereafter given 1000 units of substance P during 20 minutes' infusion. Three minutes after the start of infusion (7 units per minute) the patient got a slight bright red flush in the face, which, however, successively and spontaneously ceased and completely disappeared during continuous infusion. The rate was then successively increased to a maximal speed of about 60 units per minute. No signs of motility could be registered until 8 minutes after the start of infusion, when a few seg-

Fig. 3 A and B. Case S. S. Loop of proximal part of ileum observed during infusion of saline (3 A) and 40 units of substance P per min. (3 B). All pictures in one series are taken within 5 seconds. Series 3 B shows the development of at least two peristaltic waves (indicated by arrows). Each series of pictures should be seen from left to right.

mental contractions started, rapidly narrowing the shape of contrast and followed by adequate peristaltic waves, extending over about 10 to 20 cm of length in the aboral direction (Fig. 4). The motor activity, how ever, then became fainter even during co tinuous infusion and disappeared rapid after the end of infusion. Ten minutes lat

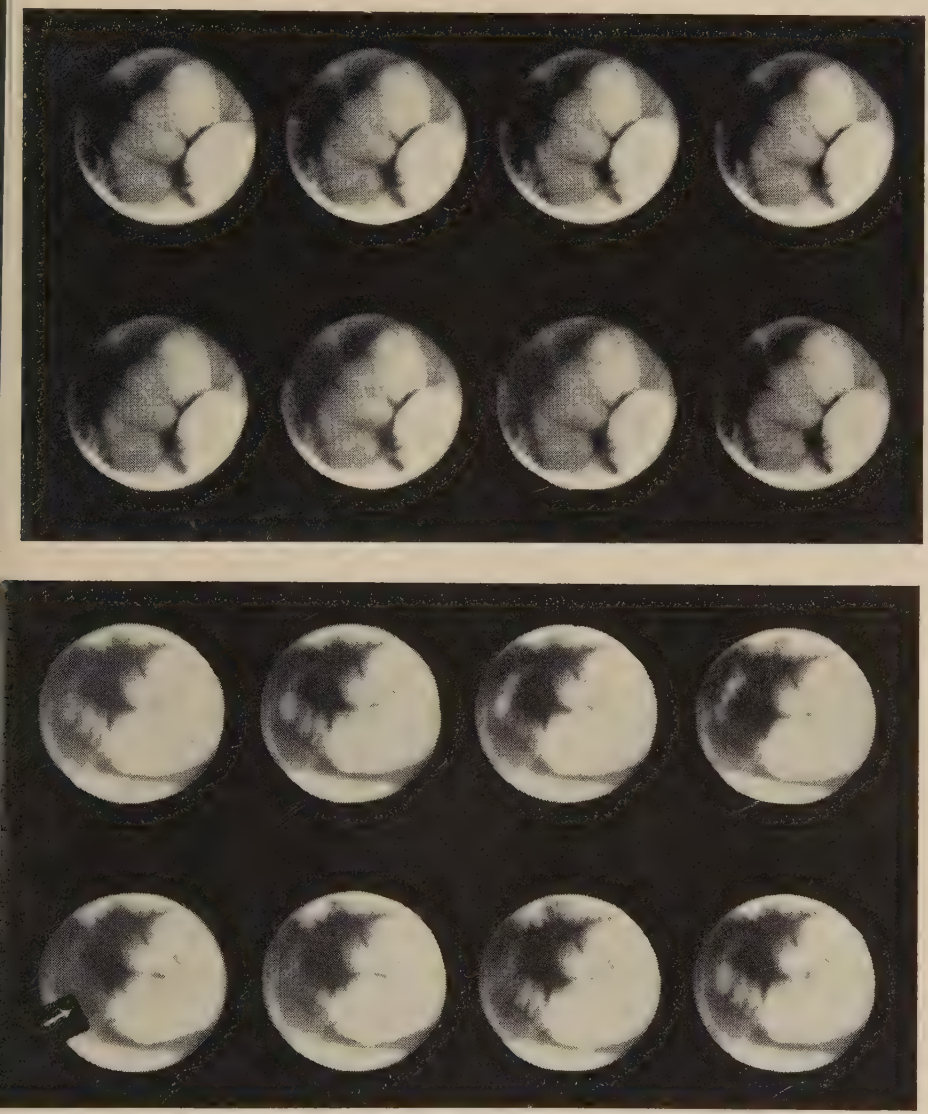

Fig. 4 A and B. Case P. B. Intestinal paralysis. Loop of proximal part of ileum. No intestinal movements observed during saline infusion (4 A). 4 B shows a period of 5 seconds during infusion of 50 units of substance P per min. Note the contracted segment to the left showing passage of contrast in the aboral direction.

t again had the same shape as before usion of substance P.

des the symptoms described above, e marked effects were observed. After completed infusion the subjects were free from symptoms and no late complications occurred in any of the cases.

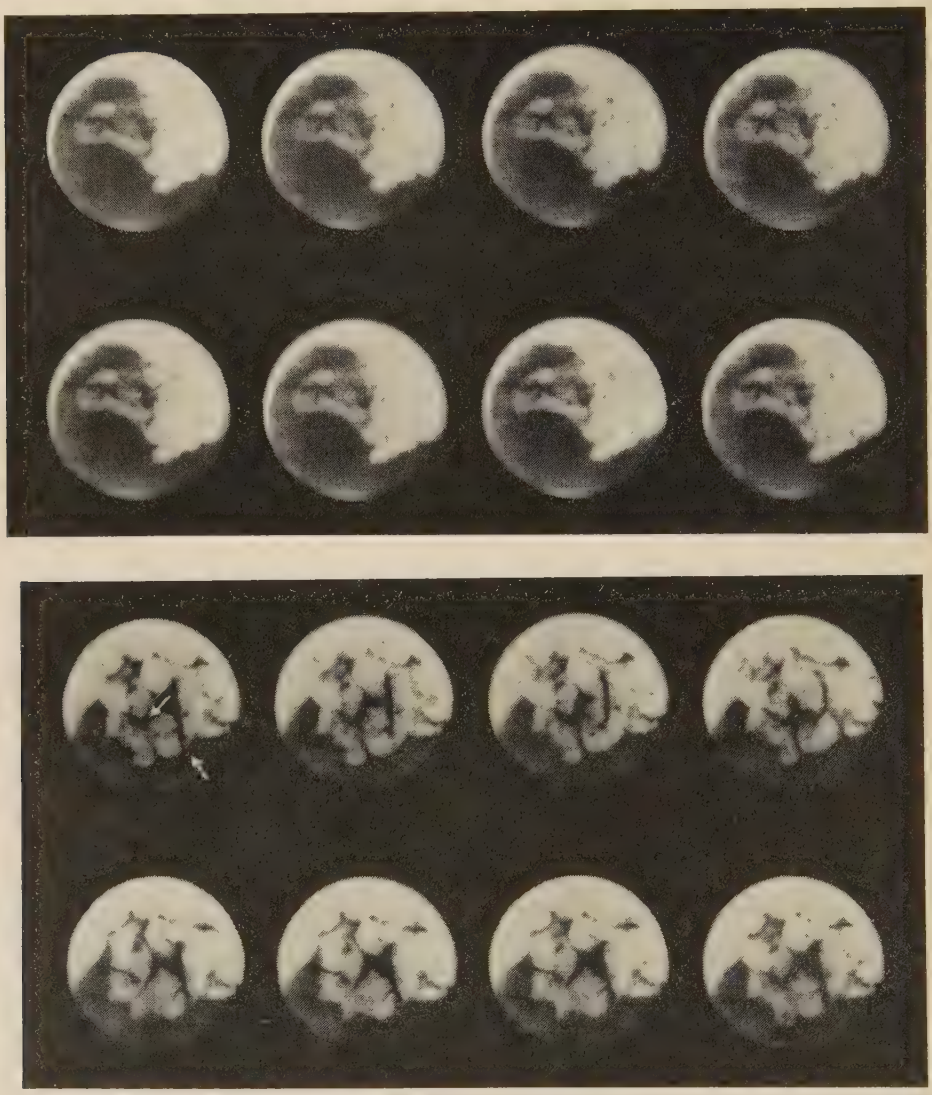

Fig. 5 A and B. Case H. S. Proximal segments of the ileum observed during infusion of saline (5 A) and prostigmin (25 μg per min.) (5 B). Increased activity in the circular muscles are seen over the whole area, dividing the contrast into small portions. No typical peristaltic waves are observed.

Prostigmin

Three of the subjects later received prostigmin by intravenous infusion, a total of 0.5 mg administered during 20 minutes. In these experiments the procedure was the same as that described for substance P. In all the cases a fully observable effect on the intestinal motility was also obtained with

nin. This effect, however, was not til about 10 to 15 minutes after the ng of infusion and lasted for at least after completed infusion. A marked in the segmental movements and a one predominated the picture, while ct on the peristaltic activity seemed ess marked than that produced by e P (Fig. 5). The subjects fre- experienced a severe griping pain in omen. No other signs and symptoms served. The pulse rate and the blood were practically unaffected.

ffect of prostigmin was also studied atient with intestinal paralysis. The of 0.5 mg of prostigmin started ne hour after the end of the substance on. Segmental contractions could also ted with prostigmin. No typical peri- novements were, however, observed. tivity rapidly disappeared after the the infusion.

DISCUSSION

use of the image intensifier makes it to follow the motility in an intestinal several hours by repeated observa- ithout subjecting the person to any total X-ray intensity than that cor- ing to some routine roentgen exa- ns. Studies of dose conditions in n cinematography with image inten- ave shown that with intestinal studies ype mentioned here the skin dose per of cineradiographic exposure amounts es below 20 roentgen units (Larsson tsson). As a comparison it may be ed that at an ordinary radiographic ation of the spine, a lateral view of bo-sacral region, will give the patient a skin dose of about 25 roentgen units according to measurements by Larsson (1956).

Through the monocular finder on the camera the appearance of the recorded image could be visually controlled during the entire filming. By this control together with the frequent screen examinations it was possible to ensure that the film most representatively recorded the current motility. The method also allows very close study of the different components of motility, by which the contraction of the segmental muscles can be distinguished from that of the longitudinal ones. The frequency, speed and extension of the peristaltic waves can also be studied.

The preparation of substance P used had such a degree of purity that it was well tolerated by the subjects. It is not chemically pure but contains in all probability biologically inactive polypeptides of low molecular weight. It is, however, biologically homogenous, and the presence of histamine, acetylcholine and 5-hydroxytryptamine could be ruled out by preliminary biological studies. Besides the increase in intestinal motility, the only observable effect was a rapidly transient peripheral vasodilatation with moderately increased pulse rate. Repeated subcutaneous injections did not produce any symptoms of anaphylactic shock. The prolonged intravenous infusion, however, was performed once only in each subject.

The substance was administered by the parenteral route since, owing to its chemical structure, it is quickly broken down by trypsin and chymotrypsin (Euler, 1936; Pernow, 1955). The effect on the intestinal motility was clearly observable after only a minute or two and remained unchanged during the whole period of infusion. After completed

administration the intestine fairly quickly resumed its ordinary activity. This is probably explained by the fact that substance P is rapidly inactivated by a polypeptidase present in the intestinal walls (Pernow, 1955; Eber & Lembeck, 1956). All types of intestinal movements seem to be activated by substance P. Segmental deep constrictions which incessantly changed place suggested a marked increase in the activity of the circular muscles, while the peristaltic waves, which travelled over several decimeters of intestine apparently carried the contrast medium in the direction of the colon.

A clear difference could be made out with respect to the effect of substance P and prostigmin on the intestinal muscles. Prostigmin influenced mostly the local segmental constrictions with the result that the contrast medium was divided into small balls separated from one another. The peristaltic activity observed after administration of prostigmin was notably less powerful than that after substance P.

Quite a distinct effect was obtained in the patient with paralytic ileus. The effect was only temporary, however, and after completed infusion of substance P the intestine resumed its initial atonic appearance. In this case the interpretation was rendered difficult by the fact that the patient also had a terminal ileitis which constituted a mechanical obstruction in the small bowel. Although this case does not permit any definite conclusions about the effect of substance P on cases with paralytic ileus, the results justify a trial of the substance in such cases where the current methods of treatment have failed to produce the desired result.

SUMMARY

The effect of substance P on intestin motility was studied in man. The substan was administered subcutaneously and by travenous infusion. The intestine contain a barium meal was studied cinematograph ally by means of an image intensifier co nected to the cinecamera.

After intravenous infusion of 600—100 units of substance P a marked increase in t segmental and peristaltic movements was o served within a few minutes and lasted u to 20 minutes after the end of the infusio

Except for a small and transient increa in the pulse rate, a decrease in the systol blood pressure and a bright red flush in t head immediately after the start of the i fusion, no side-effects could be observed.

In one case of intestinal paralysis a tra sient motility, comprising both segmental a peristaltic contraction, was initiated by inf sion of substance P.

A stimulating effect of substance P w also observed in isolated segments of huma duodenum and ileum.

The effect of substance P was in thr cases compared with that of prostigmin. T stimulating effect of prostigmin after intr venous infusion was established 10 to minutes after the infusion had started a lasted about one hour after the end of t infusion. The effect was most pronounced the segmental movements of the small i testine.

REFERENCES

Blair, M. R. & Clark, B. B.: An evaluation of action of substance P on the jejunum of rabbit. J. Pharmacol. exper. Ther. *117*, 1956.

W. W., Feldberg, W., Paton, W. D. M. hachter, M.: Distribution of the histamine substance P in the wall of the dog's ive tract. J. Physiol. *115,* 163, 1951.
& Lembeck, F.: Über den enzymatischen der Substanz P. Arch. f. exper. Path. armakol. *229,* 139, 1956.
s, T. & Pernow, B.: On the occurrence bstance P in the recto-sigmoid in Hirsch-g's disease. Acta Physiol. Scandinav. *27,* 952.
S. v.: Untersuchungen über Substanz P, ropinfeste, darmerregende und gefässer-nde Substanz aus Darm und Gehirn. f. exper. Pathol. Pharmakol. *181,* 181,

ddum, J. H.: An unidentified depressor nce in certain tissue extracts. J. Physiol. , 1931.
B.: Untersuchungen über die biologische ung der Substanz P. Acta Physiol. Scan-*3,* 270, 1942.

Janker, R.: Experiences with the Philips 5 Image Intensifier in radiological practice and scientific work. Medica Mundi *2,* 22, 1956.
Larsson, L. E.: Radiation doses to patients and personnel in modern roentgen diagnostic work. Acta Radiol. *46,* 680, 1956.
Larsson, L. E. & Mattsson, O.: To be published.
Penning, L.: The Philip Image Intensifier and its application in medical practice. Medica Mundi *1,* 6, 1955.
Pernow, B.: Substance P distribution in the digestive tract. Acta Physiol. Scandinav. *24,* 97, 1951.
— Studies on substance P. Purification, occurrence and biological actions. Acta Physiol. Scandinav. *29,* suppl. 105, 1, 1953.
— Inactivation of substance P by proteolytic enzymes. Acta Physiol. Scandinav. *34,* 295, 1955.

Scandinav. J. Clin. & Lab. Investigation 26—33, 10, 1958.

NORMAL AND ABNORMAL BLOOD CATALASE ACTIVITY IN ADULTS[1]

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(Received for publication, September 27, 1957)

At the beginning of this century it was believed that low blood catalase values, referred to unit volume of blood, were of diagnostic importance in certain diseases, but no attention had then been given to a simultaneous anemia. van Thienen (1920) pointed out that the catalase activity in blood should be related to the number of erythrocytes and suggested the use of a "Katalaseindex". For thirthy healthy subjects he found the index to be independent of sex and age. Normal index values were obtained for patients suffering from cancer, tuberculosis, acute loss of blood, severe anemia of different etiologies, and leukaemia, whereas significantly elevated values were found in pernicious anemia. A recalculation of van Thienen's figures reveals that the blood catalase activity was as an average 34 per cent higher in pernicious anemia than in normals even if hemoglobin content instead of erythrocyte number is made the basis for comparison. Dianzani (1948) reported that venesection rapidly raised the blood catalase level in guinea pigs. He used a manometric method for the assay. Richardson, Huddleson, Trustdorf & Bothea (1953) confirmed the elevated activity in pernicious anemia. Among 152 patients with vario diseases only one, with the preliminary dia nosis of "acute abdomen", had a significant lowered blood catalase activity. Yamagata Seino (1953) reported for a number of co mon diseases variations in the blood catal index and attributed diagnostic importan to them. Their results have so far not be confirmed, and some criticism can be rai against their technique. Allison & Bu (1955) found the reticulocytes in the pe pheral blood to posses a higher catalase ac vity than the mature erythrocytes, a contin ous fall occurring during the ageing of cell. Allison (1957) recently observed a ch with an atypical hemolytic anemia with reduction in the blood catalase activity one third of the normal. Takahara (19 discovered in a Japanese family a new t of constitutional abnormality, "acatalasemi in which the blood was completely devoid catalase activity. Nakamura, Yoshiya, Kaz & Kikuchi (1952) found that these patie lacked catalase in other tissues as well suggested the term "anenzymia catalase

Whereas a decrease in liver catalase a vity has been observed in man and in anim in some diseases as well as after the ad nistration of certain drugs it is thus rarely that abnormal blood catalase va

[1] A preliminary report of this investigation was given at the Third International Congress of Biochemistry, 1955.

d. The difference can be seen as a nce of the dissimilarity in activities the highly specialized bone marrow more versatile liver; in the latter tabolite might influence the forma- unction of catalase. From this inter- one might postulate that there e conditions in which the blood cata- vity as well is reduced.

METHODS

e assay of catalase, aliquots of 0.2 ml of e withdrawn from the patient's finger tip of a blood sugar pipette and diluted with an aqueous 0.04 per cent heparin solution. od from leukaemia patients a mucous regularly appeared. This was avoided ogical saline was used instead of water arin solution, a few seconds extra allowed molysis in the buffered peroxide solution. e was determined according to the method hsen, Chance, & Theorell (1947) in which ty is expressed as the velocity constant f.) in

$$k = \frac{1}{t} \ln \frac{x_0}{x_t}; \qquad (1)$$

ount of peroxide at time o, x_t after t A known volume of hemolysate is added emperature to a large volume of buffered .05 *M* hydrogen peroxide solution. Within four aliquots are transferred to flasks e sulphuric acid, and the remaining per- sequently titrated with permanganate. The e is taken as x_0. In the present work k dded volume of hemolysate has been taken erage of the three figures. The velocity was found to be linearily proportional to volume of hemolysate within the range 0 ml of hemolysate. Convenient reaction e obtained with 0.05 and 0.10 ml.[1]

following abbreviations have been used: emoglobin in grams per 100 ml of blood. Erythrocytes in millions per mm^3. Reticulocytes in *per cent* of total number

The standard deviations in a single determination in $k_{0.05}$ and in $k_{0.10}$ were determined on the basis of 21 duplicate titrations with 0.05 and 21 with 0.10 ml of hemolysate and found to be 9.0 per cent for the first and 4.4 per cent for the second volume. Since the absolute errors in $k_{0.05}$ and $k_{0.10}$ are thus nearly the same $k_{0.10}$ should contribute to k_B with twice the weight of $k_{0.05}$.

$$k_B = \frac{4}{0.15} (k_{0.05} + k_{0.10}); \qquad (2)$$

In five determinations on a normal man, performed within a few hours and including different finger punctures, pipettes, *etc.*, the standard deviation in k_B was ± 4 per cent.

For the comparison of different blood samples the catalase activity can be referred to the hemoglobin concentration as well as to the erythrocyte number. Allison & Burn preferred hemoglobin concentration because of the larger random error in the cell counting. Our results support their choice since the standard deviation in Table I is somewhat smaller with hemoglobin content than with erythrocyte number as the basis.

Hemoglobin, erythrocytes, white blood cells, reticulocytes, and serum iron were determined by the laboratory staff according to standard procedures. Hemoglobin was measured colorimetrically as HbO_2. The standard deviation between duplicate determinations of hemoglobin, including finger punctures, dilutions *etc.*, had been determined by the laboratory as ± 5 per cent.

CLASSIFICATION OF MATERIAL AND RESULTS

Healthy adults (Tables I a and I b) were randomly collected among volunteers from the staff and the students at the hospital.

Fe: Serum iron in μg per 100 ml of serum.

$k_{0.05}$: Experimentally determined rate constant in sec^{-1} per 0.05 ml of hemolysate.

$k_{0.10}$: Same for 0.10 ml of hemolysate.

k_B: Calculated constant in sec^{-1} per ml of undiluted blood.

k_B/hb: k_B per gram of hemoglobin.

k_B/rbc: k_B per million of erythrocytes.

Table Ia. *Healthy men.*

Age	Hb	RBC	$k_{0.05} \times 10^4$	$k_{0.10} \times 10^4$	k_B/hb	k_B/rbc $\times 10^4$
21	14.8	4.9	137	276	7.5	2.2
22	14.1	4.6	121	195	6.0	1.8
22	14.2	4.7	130	233	6.8	2.1
23	14.4	5.0	119	230	6.5	1.9
23	15.1	4.9	110	226	5.8	1.8
24	14.4	4.9	132	265	7.4	2.2
24	14.1	4.9	101	230	6.3	1.8
25	14.5	4.9	134	272	7.5	2.2
35	17.6	5.6	141	245	5.9	1.8
37	14.0	4.7	117	237	6.7	2.0
41	14.2	4.4	127	281	7.7	2.5
43	14.5	4.6	104	222	6.0	1.9
56	14.1	5.4	122	226	6.6	1.7
56	14.7	4.8	124	270	7.1	2.2
58	15.1	4.8	101	214	5.6	1.8
					6.6	2.0
		n = 15			±0.69	±0.23

Table Ib. *Healthy women.*

Age	Hb	RBC	$k_{0.05} \times 10^4$	$k_{0.10} \times 10^4$	k_B/hb	k_B/rbc $\times 10^4$
17	12.7	4.6	122	213	7.0	1.9
21	12.4	4.3	93	212	6.6	1.9
23	13.8	5.0	95	166	5.0	1.4
24	12.5	4.5	125	239	7.8	2.2
29	12.5	4.0	98	199	6.3	2.0
32	12.4	4.2	98	203	6.5	1.9
33	12.9	4.3	113	225	7.0	2.1
36	13.9	4.6	148	233	7.3	2.2
40	13.5	4.8	92	180	5.4	1.5
46	13.6	4.9	108	205	6.1	1.7
49	14.1	4.7	99	190	5.5	1.6
52	13.2	4.6	127	241	7.4	2.1
56	13.1	4.2	104	193	6.0	1.9
60	14.1	5.0	129	224	6.7	1.9
65	13.8	4.7	103	201	5.9	1.7
					6.4	1.9
		n = 15			±0.80	±0.25

The qualifications for acceptability as normal were a) Hb > 14.0 for men and 12.6 for women, and b) no recent history of acute disease and no chronic disease or feeling of tiredness or sickness. It may be mentioned that about one-third of all female volunteers aged 18—30 years had less than the above hemoglobin content. No influence of age upon k_B/hb or k_B/rbc is seen.

Normal catalase value in the sequel m k_B/hb-values within the range arithm mean ± 3 S. D. for healthy subjects of same sex. It is 4.5—8.7 for men and 4.0— for women. The difference is not significa

All patients but two (acute porphyria a a woman with pernicious anemia) were e mined at Karolinska Sjukhuset. Unless oth wise mentioned such cases were avoided which the characteristics of the disease co possibly have been masked by therapy, cluding blood transfusions, or by compli tions. With the exception of three cases leukosis and one with malignant eryth blastosis no patients with malignant tum are included.

The patients have been divided into following groups:

1. *Cardiovascular diseases* (Table II) moderate severity such as slight hypertensi milld arteriosclerosis or compensated *viti organicum cordis*, including a case of c genital malformation. This group showed t hospitalization in itself caused no abnorm k_B/hb-value.

Table II. *Cardiovascular diseases*

Age	Sex	Hb	k_B/h
43	M	14.2	5.3
74	M	14.8	6.6
21	M	14.6	8.2
19	M	13.8	6.7
64	M	13.5	7.1
28	F	11.9	6.6
24	M	21.8	5.1
24	F	11.9	8.9
53	M	13.7	6.7
55	M	14.1	5.6
39	F	12.1	6.8
42	M	13.9	4.9
45	M	14.5	6.5
			6.3
	n = 13		± 1.1

ble III. *Rheumatoid arthritis.*

	Sex	Hb	k_B/hb
	M	13.6	5.4
	M	14.5	6.0
	M	14.8	6.8
	F	12.9	4.9
	F	11.0	5.1
	F	11.3	6.4
	F	12.7	5.2
	F	12.2	6.8
			5.8
	n = 8		± 0.78

rheumatoid arthritis (Table III) at low although not significantly re- ılues were found. Cases with advan- nia were avoided for reasons given was recently reported by Alexander, nd, Royh & Duthie (1956) that the of the erythrocytes is shortened in ases of rheumatoid arthritis. This ance ought to elevate the catalase (Allison & Burn, 1955).

ven patients with *liver cirrhosis* rent types showed normal values IV).

nuine, untreated and uncomplicated *nic anemia* (Table V a) was found three women. Numerous other pa-

Table IV. *Liver cirrhosis.*

	Sex	Hb	k_B/hb
	F	11.6	6.1
	F	10.3	5.5
	F	12.4	7.0
	M	13.1	8.0
	M	11.5	6.6
	M	12.7	6.1
	M	13.2	6.7
			6.6
	n = 7		± 0.80

Table V a. Sideropenic anemia.

Age	Sex	Hb	RBC	Ret.	Fe	k_B/hb	k_B/rbc × 10^4
26	F	10.1	3.5	10	18	4.7	1.3
43	F	6.2	2.1	26	18	6.0	1.7
22	F	9.7	3.5	10	18	6.4	1.8

tiens with anemia and low serum iron values were considered but for all of them either some disease causing the anemia or some kind of iron therapy prevented inclusion in this group. The three showed normal catalase values. For the sake of comparison values are also given (Table V b) for eight persons who appeared and felt quite healthy and volunteered as normals but showed too low hemoglobin values. Also in this group normal catalase activity was found.

5. Five patients with *hemolytic anemia* gave normal values. (Table VI).

6. Four patients with pernicious anemia were studied before, during, and after treatment with vitamin B_{12}. Three of them were

Table V b. *Apparently healthy adults with low hemoglobin values.*

Age	Sex	Hb	RBC	k_B/hb	k_B/rbc × 10^4
21	F	11.3	4.0	8.2	2.3
22	F	12.2	3.9	8.0	2.5
24	F	10.7	3.9	8.9	2.4
27	F	11.7	4.2	7.7	2.1
28	F	11.5	3.8	6.3	1.9
31	F	10.8	3.8	5.4	1.5
24	M	13.3	4.3	6.6	2.0
36	M	13.6	4.5	6.2	1.9
				7.2	2.1
	n = 8			± 1.20	± 0.33

Table VI. *Hemolytic anemia.*

Age	Sex	Hb	Ret.	RBC	k_B/hb	k_B/rbc × 10^4
69	F [1]	5.5	1.6	1.3	6.6	2.3
61	F [1]	4.6	2.0	-	5.2	2.0
		7.8 [3]	2.5	4.9	6.6	2.0
16	F [2]	9.3	3.0	10.0	7.7	2.4
53	F [1]	4.6	1.5	9.4	6.9	2.1
58	F [1]	9.0	2.2	4.2	7.6	3.1

[1] Acquired hemolytic anemia, *causa ignota.*
[2] Atypical congenital hemolytic anemia.
[3] Three months after first determination.

typical cases of addisonian pernicious anemia, the fourth (T. C.) had a megaloblastic anemia after resection of the stomach. The results are collected in Fig. 1. Although there are considerable fluctuations in the values, the general tendency is obvious. S. B. was given antibiotics prior to vitamin B_{12}, and the resulting slight response in her reticulocyte count may have influenced the catalase values. It can be seen, particularly in A. W. and T. C., that the change from megaloblastic to normoblastic hematopoiesis reduces the k_B/hb-ratio to the normal level.

No increase in the catalase activity could be detected at the time of maximal reticulocyte count. In consideration of the total number of determinations that were made on reticulocyte-rich blood specimens it seems unlikely that a peak or even shoulder in the catalase values could have been masked by the random error in the method, at least if the reticulocytes contain three times more catalase than the mature cells. Our result on this point is at variance with the conclusions drawn by Allison & Burn (1955) from their case of pernicious anemia. In rabbits, made anemic by means of acetylphenylhydrazine,

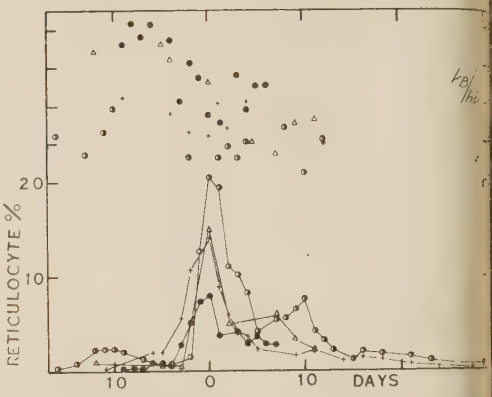

Fig. 1. The catalase-hemoglobin ratio in blood four patients with pernicious anemia before, dur and after the reticulocyte peak.
◑ S. B. woman, age 80. ● T. C. man, age 6
+ E. B. woman, age 74. △ A. W. man, age

we found the reticulocyte to posses 3– times the catalase activity of the mat erythrocyte. There may be a difference tween the reticulocytes in the two hema poietic courses. .It should be pointed out, that the present investigation has dealt w the apparent catalase activity in laked wh blood rather than the content of catalase the cells.

7. The remaining patients formed a hete genous group, consisting of 24 men and women, aged 26—74 years and suffer from some dieseases seen in a medical cli (diabetes, nephrosis, postinfectious anem lupus erythematosus, polycythemia, ac porphyria, hypothyreosis, acute pancreati reticulosis, dysfunction of the bile ducts, ac pericarditis, exophthalmus, duodenal ul coronary thrombosis, ulcerative colitis, ma nant erythroblastosis, malignant lymphog nulomatosis, and leukemia). No more th three patients with the same diagnosis w studied, and it will suffice to mention t

VII. *Distribution of k_B/hb-values 7 (see text). The four cases of cious anemia are not included in this table.*

4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	> 8.9
2	3	12	3	3	0
0	5	2	2	2	0

alues were found in all cases with tions commented below. The distri- all values in group 7 is given in I.

nen with polycythemia vera were One had received no treatment dur- receding six months, and his general , blood composition, and catalase ere normal. The other one had been C of P^{32} six weeks before the cata- rmination, and had responed with a Hb from 18.4 to 15.8. k_B/hb was 8.8. oteworthly that a man with malig- hroblastosis gave the normal ratios .5 in six determinations during four

llowing patients gave values outside e for normals.

G. 320103. Woman, age 24, com- mitral stenosis.= 8.9 (*cf.* Table II). . P. 350226. Male outpatient, 21 th a preliminary diagnosis of peri- k_B/hb = 8.9.

A. R. A. 940908. Man, 62 years. emia, k_B/hb = 8.8 (*cf.* above).

. F. 09. Man, 47 years. Rheumatoid + bronchopneumonia + anemia, se- 22 μg. k_B/hb = 4.4.

four values are just outside the ange, and no further investigations ar been made on the patients.

(v) A. G. T. 406/1955. A 30 year old woman was admitted to the hospital in August 1955 because of intermittent and rather severe pain of spastic type in the right hypochondrium. Spasmolytics gave some relief. Gall bladder disease was suspected but cholecystography was normal. The pathological findings were a consistent thrombocythemia (900 000—1 200 000 per mm^3 blood), some increase in number and size of megacaryocytes in sternal and iliac marrow, slight enlargement of liver and spleen, and occasionally subfebrility and moderate leukocytosis (15 000 per mm^3). Biopsies of liver and spleen revealed normal histology, and radiographs of stomach, duodenum, small intestine, colon and kidneys were likewise normal. Percutaneous splenoportography, liver function tests, blood volume and total hemoglobin determinations also failed to show anything abnormal. The porphobilinogen and porphyrin tests in the urine were consistently negative. No conclusive support for a diagnosis of incipient polycythemia or chronic granulocytic leukemia was obtained. Her pains were tentatively attributed to a biliary colic, possibly in connection with a slight infection in the biliary ducts and gall bladder. The abdominal symptoms have gradually vanished, and she felt quite well in August 1957.

Repeated determinations on her blood during two years have consistently shown low ratios, k_B/hb = 2.7—3.2. Separate determinations were made (August 1957) on plasma and erythrocytes from heparinized blood. Whole blood gave $k_B = 0.33$ but a hemolysate of saline-washed erythrocytes 0.45 when referred to the same hemin concentration. An aliquot of a 1 : 100 dilution

with phosphate buffer pH 7 of a solution of crystallized horse liver catalase gave $k = 0.0177$ sec^{-1}, whereas after dilution with plasma in the same proportion $k = 0.0122$ sec^{-1} was obtained. Since this difference is about the same as the one found between erythrocytes and whole blood it is possible that the patient's plasma contains some substance which can inhibit catalase. Blood specimens from three normal men were examined in the same way but their plasma did not inhibit catalase, nor was there any difference in catalase activity, expressed as k_B/hb, between laked erythrocytes and whole blood hemolysate.

(vi) H. S. 021129, MP 1955. A 53 year old man appeared at the outpatient department (Dec. 1955) and complained about lumbar pain of apparently moderate intensity and some oppression in the epigastric region and left hypochondrium. No pathological laboratory data were obtained in the routine examination. X-ray examination disclosed a disc degeneration in the lumbar region and a calculus in the right ureter. Kidneys and spleen were of normal size and location. There was no sign of impaired renal function. On this occassion he had $Hb = 12.8$ and $k_B/hb = 2.5$. In February 1957 he spontaneously appeared at the outpatient department complaining of tiredness and spells of diarrhea. X-ray of the colon, fecal examination, proctoscopy, and liver function tests revealed nothing abnormal. k_B/hb was 2.7. At the end of March the quotient was unchanged, 2.8. An irritable colon was likely to be the cause of his complaints. In August 1957 he was again seen at the outpatient department. He felt fairly well, and only routine determinations on his blood were made. $Hb = 12.3$ and $k_B/hb = 2.8$. No c lase inhibition by his own plasma could demonstrated.

COMMENTS

The aim of the present investigation initially to find out a) how frequently normal blood catalase values occur in a r domly chosen hospital material, and whether any irregularities found can be ferred to a certain disease or family diseases.

The answer to the first question must given with reservation for the fact that s selection of the material was made towar the end of the study, when some rare d eases or statistically "needed" cases were p ferred to trivial ones. It seems to be nec sary to follow the patients V. G., O. A. R. A., and B. F. for some time bef an answer is given.

As regards the second question the inve gation has to some extent confirmed Thienen's observation that pernicious anem is characterized by a high catalase-hemog bin ratio. The decrease in connection with reticulocyte peak in patients T. C. and A. was remarkably fast.

H. S. has clinically, without any dou been considered as an uncomplicated case an irritable colon. Because of the random discovered low catalase activity a query provisionally put after the diagnosis. A. G. is by no means a clear case from a clini point of view, and the diagnosis of throm cytemia + abdominal pains of unknown gin will obviously permit several etiolog There is no support in the histories of H. and A. G. T. or in the laboratory findi for an assumption that they represent ferent stages in the same disease.

SUMMARY

blood catalase activity has been
l as the rate constant in sec^{-1} per
hemoglobin (k_B/hb) for the cata-
composition of hydrogen peroxide,
under standardized conditions.
ib has been determined for fifteen
each sex and found to be 6.6 ± 0.69
or men and 6.4 ± 0.80 for women.
ithin the range (average ± 3 S. D.)
n considered normal.
erminations were made on eighty-
nts in a hospital department for in-
dicine. Normal values were found
y-four of them, representing a num-
ted diseases.
r patients gave values just outside
al range (8.9, 8.9, 8.8, 4.4). They
yeat been further investigated.
nificantly reduced values were found
n (2.5—2.8) and one woman (2.7—
eir values have remained almost
during a period of nearly two years.
an's blood plasma seemed to contain
that inhibited catalase. The two
probably represent different nosolo-
ities.
r patients with pernicious anemia
owed during the course of the treat-
h vitamin B_{12}. Two of them showed
high k_B/hb-values, which were re-
normal level within one or two
he most rapid decrease occurred at
for reticulocyte maximum or 1—3
ier. In the other two cases of per-
nemia the initial catalase level was
y normal, and no pronounced
occurred during the treatment. No
increased catalase-hemoglobin ratio
appeared simultaneously with the reticulocyte maximum.

7. The investigations are being continued.

ACKNOWLEDGEMENTS

Miss Anna Hartkoorn gave valuable assistance during the work. K. G. P. received a grant from *Statens medicinska forskningsråd.*

REFERENCES

Alexander, W., Richmond, J., Royh, H., & Duthie, R.: Nature of anemia in rheumatoid arthritis. II. Survival of transfused erythrocytes in patients with rheumatoid arthritis. Ann. Rheumat. Dis. *15,* 12, 195.

Allison, A. C.: Acute haemolytic anaemia with distortion and fragmentation of erythrocytes in children. Brit. J. Haematol. *3,* 1, 1957.

Allison, A. C., & Burn, G. P.: Enzyme activity as a function of age in the human erythrocyte. Brit. J. Haemathol. *1,* 291, 1957.

Bonnichsen, R. K., Chance, B., & Theorell, H.: Catalase activity. Acta Chem. Scandinav. *1,* 685, 1947.

Dianzani, M. U.: Le modificazioni della catalasi ematica in cavie sottoposte a salassi. Boll. soc. ital. biol. sper. *24,* 1280, 1948.

Nakamur, H., Yoshiya, M., Kaziro, K., & Kikuchi, G.: On "Anenzymia Catalasea", a new type of constitutional abnormity. Proc. Japan. Academy, *28,* 59, 1952.

Richardson, M., Huddleson, I. F., Trustdorf, M., & Bothea, R.: Study of catalase in erythrocytes and bacteria. III. Catalase activity of erythrocytes from humans and animals in various pathological states. Arch. Biochem. *47,* 338, 1953.

Takahara, S.: Acatalasemia (Lack of catalase in blood) and an oral progressive gangrene. Proc. Japan. Academy *27,* 295, 1951.

van Thienen, G. J.: Ueber die perniziöse Anemie als eine selbständige Krankheit. Deutsch Arch. Klin. Med. *131,* 113, 1920.

Yamagata, S., & Seino, S.: Studies on blood and plasma catalase. Tokohu J. Exper. Med. *57,* 101, 231, 239, 249, 1953.

Scandinav. J. Clin. & Lab. Investigation 34—38, 10, 1958.

ON THE CLINICAL VALUE OF PAPER ELECTROPHORESIS AND OF DETERMINATION OF MUCOPROTEIN IN ASCITIC FLUID

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(Received for publication October 2, 1957)

There are only a few publications concerning protein electrophoresis on ascitic fluid, but none of diagnostic significance (Luetscher, 1941; Macheboeuf, Reyberotte & Brunerie, 1951; Wunderly & Wuhrmann, 1953; De Wael, 1956; Combrisson, Debray & Housset, 1957). An investigation of the mucoproteins in ascites was reported recently (Taipale & Hokkanen, 1956).

The aim of this report is to give some preliminary data on quantitative and qualitative differences between ascitic fluids in various clinical conditions. The results obtained hitherto, seem to indicate that protein analysis of ascitic fluid is of diagnostic value.

MATERIAL AND METHODS

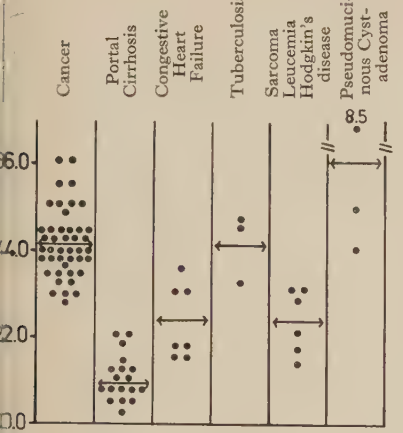
The material comprises 82 cases, in which the diagnosis appeared to be well established. As a result of the clinical and the pathological findings the material could be classified as follows: Cancer (43 cases), portal cirrhosis (18), congestive heart failure (7), tuberculosis (3), a mixed group with sarcoma, leukemia and Hodgkin's disease (6), pseudomucinous cystadenoma (3), and nephrotic syndrome (2).

In most cases the paracenthesis was a primary one. The following determinations were performed in duplicate on the centrifugate, as well as on a serum sample collected simultaneously. *Total protein* was determined with the biuret method, *mucoprotein* according to Winzler, Devor, Mehl & Smyth (1948). For the *filter paper electrophoresis* of the proteins the apparatus and technique given in Goa's modification (1955) was used. The *albumin/globulin ratio* (Alb./Glob.) was calculated from the paper strips, classifying the α_1- and α_2-fractions to the globulins. At a low protein level the ascitic fluid was concentrated by means of dialysis against a dextran, Macrodex ®(Schneider & Wallenius, 1951), by ultradialysis under high pressure (Esser & Heinzler, 1952) or by lyophilization. In lactescent ascitic fluid most of the lipids were removed by centrifugation at 15 000 r.p.m. (approximately 25 000 g).

RESULTS

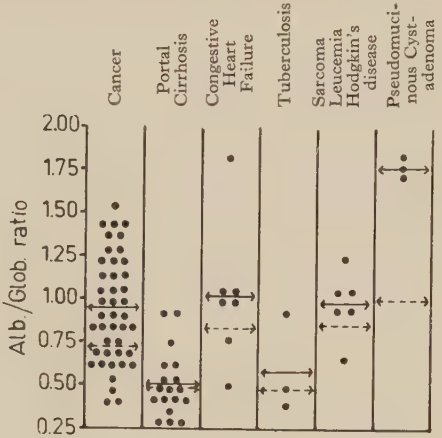
The results are listed in Figs. 1—4. Every dot represents the value for a single ascitic fluid. The mean value of the dots in a group is marked by a line. In Figs. 2—4 also the corresponding means for serum are recorded (— — — —). The two cases of nephrotic syndrome are not included in the figures because of the specific composition of the ascites. They are reported separately.

Total protein (Fig. 1). The amount of total protein in ascites ranged from 0.4—8 g per 100 ml. It was significantly higher in the cancer group than in the congestive heart failure group ($P < 0.001$). The latter group had a significantly higher amount of protein than the portal cirrhosis group ($P < 0.00$). The cases of tuberculosis had values near



	Cancer	Portal Cirrhosis	Congestive Heart Failure	Tuberculosis	Sarcoma Leucemia Hodgkin's disease	Pseudomucinous Cystadenoma
an	4.2	1.0	2.4	4.1	2.4	6.1
D.	0.84	0.45	0.97	0.78	0.78	2.31

Fig. 1. Total protein in Ascites.

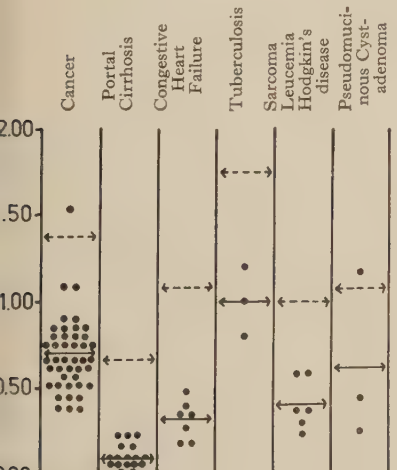


	Cancer	Portal Cirrhosis	Congestive Heart Failure	Tuberculosis	Sarcoma Leucemia Hodgkin's disease	Pseudomucinous Cystadenoma
Mean	1.0	0.5	1.1	0.6	1.0	1.8
S. D.	0.28	0.20	0.41	0.20	0.20	0.17

Fig. 3. Alb./Glob. ratio in Ascites.
For explanation see text.

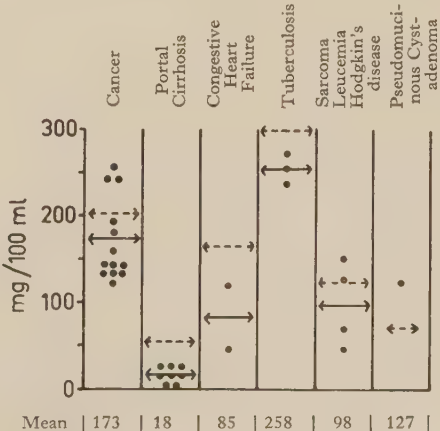
the mean of the cancer group, the group was found in the same range ases of congestive heart failure. One pseudomucinous cystadenoma had a gh amount of total protein (8.5 g per). Concerning the amount of total protein in serum, there were no remarkable differences between the groups, nor was there any correlation between the amount of total protein in serum and in ascites in the material as a whole.

($\alpha_1 + \alpha_2$)-globulin (Fig. 2). With the electrophoretic method used, the α_1-globulin was well separated from the albumin fraction.



	Cancer	Portal Cirrhosis	Congestive Heart Failure	Tuberculosis	Sarcoma Leucemia Hodgkin's disease	Pseudomucinous Cystadenoma
ean	0.7	0.1	0.3	1.0	0.4	0.6
D.	0.21	0.05	0.12	0.21	0.15	0.49

g. 2. ($\alpha_1 + \alpha_2$)-globulin in Ascites.
For explanation see text.



	Cancer	Portal Cirrhosis	Congestive Heart Failure	Tuberculosis	Sarcoma Leucemia Hodgkin's disease	Pseudomucinous Cystadenoma
Mean	173	18	85	258	98	127
S. D.	47.1	8.6	—	16.0	49.9	—

Fig. 4. Mucoprotein in Ascites.
For explanation see text.

The variations of the usually small α_1-fraction almost followed those of the α_2-globulin. These two fractions were therefore considered as a whole-($\alpha_1 + \alpha_2$)-globulin. The absolute concentration in the cancer group was significantly higher than in the heart failure group ($P < 0.001$); this latter group in its turn had a significantly higher amount of ($\alpha_1 + \alpha_2$)-globulin than the portal cirrhosis group ($P < 0.001$). The mean value for the three cases of tuberculosis was higher than in the cancer group. The absolute as well as the relative amount of ($\alpha_1 + \alpha_2$)-globulin in ascites was in all cases lower than in serum.

Albumin, β- and γ-globulin. The portal cirrhosis group had a high content of β- and γ-globulin. The other groups did not show any systematic differences, hence they are not recorded here.

Alb./Glob. ratio (Fig. 3). The three cases of cystadenoma had a high ratio. The ratios in the other groups were lower. The portal cirrhosis group had the lowest mean value with a significant difference from the cancer group ($P < 0.001$). In all cases the ratio was lower in serum than in ascitic fluid.

Mucoprotein (Fig. 4). All eight cases of portal cirrhosis tested had a very low content of mucoprotein, lower than any other case. The difference from the cancer group was statistically significant ($P < 0.001$). The three cases of tuberculosis had the highest mean amount. The values for the serum mucoproteins were in nearly all cases slightly higher than for ascites.

Nephrotic syndrome ascites. The amount of total protein was extremely low, 0.10 and 0.20 g per 100 ml, respectively. After concentration of the ascites the electrophoretic pattern had quite a typical appearance, with

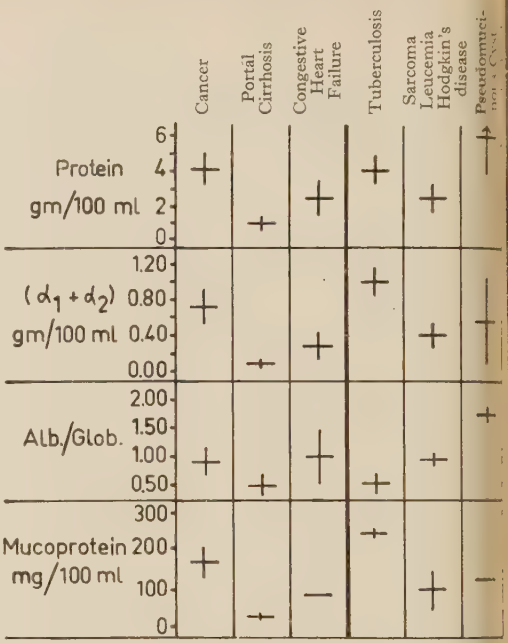


Fig. 5. Survey and S. D. of the mean values i
Ascites. The vertical lines indicate ± 1 S. D.

great α_2- and β-globulin fractions, resemblin those in serum. The amount of mucoprote was fairly high in the one case determine (113 mg per 100 ml ascites, and 226 m per 100 ml serum, respectively).

DISCUSSION

As pointed out in the introduction, the ai of this investigation was to evaluate the pr tein analysis of ascitic fluid as a clinical te Hence the results are only discussed from th point of view of differential diagnosis. T results are summarized in Fig. 5.

Some of the groups are too small to perm any conclusions. Still, they need attenti when classifying ascites from cases of que tionable diagnosis by protein analysis. T larger groups, *cancer, portal cirrhosis,* a *congestive heart failure* show characteris

ces in some respects. However, the l deviation of each component deter- s usually great. Consideration must aken to the influence the duration of may have on the protein composition. he results of this preliminary in- on must be judged very carefully.

eboeuf, Reyberotte & Brunerie after analysing only a few cases, d that the electrophoretic pattern same in ascites as in serum. The investigation, on the contrary, shows relative amount of albumin in prac- very case is higher in the ascitic hich is reflected in its higher Alb./ tio. This finding, however, is of no ic value.

er, the simultaneous determinations ins in serum and ascites show that the differences between the compo- termined in the various groups, are cant in serum and pronounced in Kay (1954) analyzed only serum tients with ascites. The results now contradict the value of analysing um as a routine procedure in most ith ascites; in fact, the advantage of nalysis seems to be so apparent that m determinations can be omitted.

rding the different tests on ascites, rmination of *total protein* is a simple re, giving a quick and good orienta- ut the nature of the disease. On the a high content indicates a malignant or possibly inflammatory. A low con- aks in favour of portal cirrhosis or flammatory disease. The author can- ee with the opinion, expressed by sson, Debray & Housset (1957), who small material failed to find any systematic difference between ascitic fluids of cancerous and cirrhotic origin. The differentiation of the cases is further underlined by the determination of $(\alpha_1 + \alpha_2)$-*globulin*. In addition, the absolute amount of $(\alpha_1 + \alpha_2)$-globulin seems to be rather unaffected during the course of the disease, when the other protein components usually decrease. This has been observed on repeated paracenthesis in cases of cancer. The other groups are under study.

The value of the *Alb./Glob. ratio* must be judged carefully. The different ratios found may be interpreted as characteristic of the various diseases, but they might also be an effect of a more prolonged course of the diseases, leading to disturbed protein metabolism. Only the portal cirrhosis group had a pronounced low ratio, depending on the great β- and γ-globulin fractions.

The variation of the amount of *mucoprotein* approximately followed that of the $(\alpha_1 + \alpha_2)$-globulin, but the number of observations is few. Thus far, nothing can be said about the value of this part of the investigation. The differences observed, however, agree with the findings of Taipale & Hokkanen (1956).

De Wael (1956) mentioned the usefulness of paper electrophoresis in cases of ascites. He emphasized, without giving any figures, that cases of *cirrhosis* of the liver readily can be distinguished from those of poor condition of the heart, abdominal tumors *etc.*, through their large and characteristic β- and γ-globulin fraction. Most of our cirrhotic cases showed, as mentioned above, the same appearance, but the minimal $(\alpha_1 + \alpha_2)$-globulin fraction was still more characteristic. The combination with a very low amount of both

total protein and mucoproteins confirms the diagnosis. From Fig. 5 it is further seen that if the level of the total protein content is above 4.2 g per 100 ml, the ($\alpha_1 + \alpha_2$)-globulin more than 0.7 g per 100 ml, and the content of mucoproteins above 170 mg per 100 ml, this speaks strongly in favour of a peritoneal *carcinosis* (the figures represent the mean values found). *Congestive heart failure* causes ascites with protein values between the two mentioned groups. With a certain accuracy there are, however, chances of excluding the other two main groups in the differential diagnosis.

The results now obtained from protein analysis of ascitic fluid agree with Luetscher's findings with the Tiselius apparatus (1941). However, he reported only 8 cases of cancer, congestive heart failure or portal cirrhosis. In the differential diagnosis it may, as seen from the figures for our smaller groups, even be necessary to consider other diseases for each level of the proteins determined. No definite guidance can be given. It is necessary to observe more cases.

SUMMARY

82 cases of ascitic fluid of different origins have been examined on the content of total protein and mucoprotein. Paper electrophoresis was carried out and special consideration was taken to the amount of the ($\alpha_1 + \alpha_2$)-globulin fraction. Simultaneous determination of the corresponding serum components showed these to be of less importance clinically. The differences between portal cirrhosis, congestive heart failure and cancer are emphasized for ascites. The results show that protein analysis according to the methods used is of value when establishing the clinical differential diagnosis.

The investigation is proceeding and further details will be published elsewhere.

REFERENCES

Combrisson, A.-G., Debray, J. & Housset, J. D'un test caractérisant l'ascite d'origine cirrhotique. Presse méd. *65,* 1227, 1957.

Esser, H. & Heinzler, F.: Eine Methode zur Gewinnung der Proteine aus Liquor cerebrospinalis und anderen schwach Eiweisshaltigen Lösungen durch Filtration mit Überdruck für die Elektrophorese in Filterpapier. Klin. Wchschr. *30,* 600, 1952.

Goa, J.: On Protein-bound carbohydrates in human serum. Scandinav. J. Clin. & Lab. Investigation Suppl. *22,* 1955.

Kay, H.: The value of paper electrophoresis of serum proteins in diagnosis of ascites. Brit. Med. J. *II,* 1025, 1954.

Luetscher, J. A., Jr.: Electrophoretic analysis of the proteins of plasma and serous effusions. J. Clin. Investigation *20,* 99, 1941.

Macheboeuf, M., Rebeyrotte, P. & Brunerie, M. Applications aux sérums pathologiques, aux urines et aux liquides d'ascite (Néphrose lipoidique, myélome multiple, cirrhose de Laennec) de la méthode de microélectrophorèse sur papier. Bull. Soc. chim. biol. *33,* 1543, 1951.

Schneider, G. & Wallenius, G.: Electrophoretic studies on cerebrospinal fluid proteins. Scandinav. J. Clin. & Lab. Investigation *3,* 14[illegible] 1951.

Taipale, E. & Hokkanen, E.: The mucoprotein levels of the ascitic and pleural fluids and their clinical significance. Acta med. scandinav. *15*[illegible] 113, 1956.

De Wael, J.: Application of paper electrophoresis to the differential diagnosis of canine diseases. Paper Electrophoresis. A Ciba Foundation Symposium. J. & A. Churchill Ltd., London 1956, p. 25.

Winzler, R. J., Devor, A. W., Mehl, J. W. & Smyth, I. M.: Studies on the mucoproteins of human plasma. J. Clin. Investigation *27,* 60[illegible] 1948.

Wunderly, Ch. & Wuhrmann, F.: Kapillar-permeabilität und Bluteiweisstoffe. Plasma *1,* [illegible] 1953.

. J. Clin. & Lab. Investigation 39—41, 10, 1958.

E ELECTROPHORESIS AS A MEANS OF ESTABLISHING NUMBER OF ACIDIC GROUPS IN PORPHYRIN PIGMENTS

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(Received for publication October 2, 1957)

he past few years a series of new s for the separation of porphyrins has roduced. Accordingly new knowledge e porphyrins from different biological has arisen, showing that a series of ins different from protoporphyrin, rphyrin and uroporphyrin in the of acidic groups is formed and ex- nder normal and pathological con-

amounts of these porphyrins as com- with the main porphyrins, proto-, and uroporphyrin, are very small, r, and therefore the only method for ction and identification of these por- has been the paper chromatographic s of Nicholas & Rimington (1949, r Eriksen (1953).

e methods are said to separate the ins according to the number of acidic However, it is quite obvious that the s are incapable of separating porphy- h a certain number of acidic groups rphyrins with the same chromato- behavior due to other properties of ecules.

it would be of great importance to r micro methods for the identification umber of acidic groups in a given in, and it seemed likely that zone electrophoresis should be convenient for such a separation. A couple of years ago we therefore tried to separate porphyrins from different sources by paper electrophoresis, trying a series of different types of filter paper, but we soon found that paper electrophoresis could not be used as the adsorbtion of the porphyrins, especially those with a number of acidic groups of four or less, was so high that no separation of these porphyrins was achieved.

The adsorbtion to the paper decreased with increasing number of acidic groups, but even in the case of uroporphyrin the adsorbtion was so marked that the moving spot took the form of a comet becoming more and more elongated as the spot travelled along the paper during the electrophoresis.

These observations are in accordance with the results of With (1955), but in contrast to those of Heikel (1955).

As has been shown by R. Weber (1953) the adsorbtion of a compound to the supporting medium has a marked influence on the travelling velocity of the compound in zone electrophoresis. The velocity increases with increasing concentrations of the compound. This observation was confirmed by us. Actually we found that in the case of porphyrins with 3 and 4 acidic groups we

could invert the travelling velocity of the two compounds by increasing the amount of the porphyrin with 3 acidic groups or by decreasing the amount of the porphyrin with 4 acidic groups. Thus if paper electrophoresis is used for the identification of the number of acidic groups in a porphyrin, one has to use identical concentrations of the porphyrin investigated and the markers. In this case the front of the cometlike spots will move with speeds directly comparable with the number of acidic groups.

It is quite obvious that under these conditions paper strip electrophoresis is not very useful, and in many case could be used only for porphyrins with 5 or more acidic groups. We therefore tried the method of Papastamatis & Kench (1952) using agar gel as the supporting medium and M/15 phosphate buffer pH 8.0 and found that no or very slight adsorbtion could be seen. However, the method of preparing the agar gel was rather laborious and time-consuming, and the apparatus used in many ways unfit for the purpose, especially when micro amounts of porphyrins were to be investigated. We therefore tried to work out another method, using plastic troughs instead of the water-cooled glass bridge used by Papastamatis & Kench. A series of supporting media (starch paste, starch gel, paper powder, glass powder and others) were tested. However, so far we have found that the best supporting medium is an agar gel prepared as follows.

METHODS

Agar, of the highest possible quality obtained in the market, was dissolved in the veronal buffer used (ionic strength 0.05, pH 8.6) on a water bath. All insoluble material was spun down by hard centrifugation for some minutes while the solu was still hot. By this method we got a comple clear and colorless solution.

The concentration of the gel should be as as possible. Usually we use a concentration of per cent when the apparatus is placed in the c room, and a 1 per cent agar is used when no coo device is used.

The solution was poured into the trough u a layer of the desired thickness was obtained a allowed to gel. The gel was connected with electrolyte compartments through filter pa bridges as in a usual settup for paper elec phoresis.

The porphyrins were applied in highly conce trated solution into small holes in the gel fi made by suction with Pasteur pipettes.

The trough was covered with a glass plate a a voltage applied across the agar bridge.

The height and width of the trough can varied according to the analysis to be performe however, the length must be at least 50 cm.

Usually we use a trough 50 cm long, 18 cm w and 1.5 cm high.

The whole apparatus is put into the cold ro (temperature + 4° C) during the electrophore to prevent the gel from liquefying.

When a voltage of 300 volts and a film thickn of 2—5 mm is used, a complete separation of p phyrins with from 8 to 3 acidic groups is obtai in 10 to 12 hours (see Fig. 1).

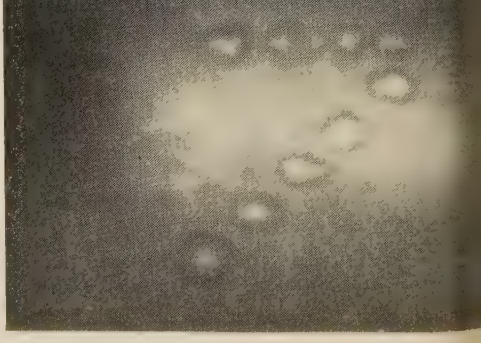

Fig. 1. Electropherograms of single samples a mixture of porphyrins which, according to t paper chromatographic behavior, have 8 6, 5, 4, and 3 acidic groups.

ectropherograms could be developed either illumination with Woods light, or an im- d be made simply by rollling a paper strip e surface of the agar film avoiding air etween the gel and the paper. The evenly paper is taken off and inspected in ght.

COMMENTS

nethod described is very suitable for g whether a porphyrin obtained from source by different methods actually expected number of acidic groups as l by paper chromatography, using line methods of Nicholas & Riming- Eriksen, which will be shown in a communication, can not be relied hen using porphyrin preparations logical sources.

REFERENCES

Eriksen, L.: Paper chromatography of porphyrin pigments. Scandinav. J. Clin. & Lab. Investigation *5,* 155, 1953.

Heikel, T.: Paper electrophoresis of porphyrin pigments. Scandinav. J. Clin. & Lab. Investigation *7,* 347, 1955.

Nicholas, R. E. H. & Rimington, C.: Qualitative analysis of the porphyrins by partition chromatography. Scandinav. J. Clin. & Lab. Investigation *1,* 12, 1949.

Nicholas, R. E. H. & Rimington, C.: Paper chromatography of porphyrins: some hitherto unrecognized porphyrins and further notes on the method. Biochem. J. *48,* 306, 1951.

Papastamatis, S. C. &q Kench, J. E.: Ionophoretic separation of porphyrin pigments. Nature *170,* 33, 1952.

Weber, R.: Zur Theorie elektrophoretischer Trennverfahren auf porösen Trägern. Helvet. chim. Acta *36,* 424, 1953.

With, T.: Paper electrophoresis of porphyrins. Scandinav. J. Clin. & Lab. Investigation *8,* 113. 1956.

Scandinav. J. Clin. & Lab. Investigation 42—45, 10, 1958.

PEPTIDES ISOLATED FROM THE SERUM OF CASES OF LEUKEMIA

By G. HAMMARSTEN and B. M. SANDELL

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(Received for publication October 9, 1957)

Chromatographic studies of the amino acids in the serum of 2 cases of di Guglielmo's erythroleukemia revealed a peptide giving a purple spot above alanine. This peptide was also found in the serum of 3 cases of myeloic leukemia, but could not be found either in the serum of 50 healthy adults or in the serum of 8 cases of Addisonian anemia. The term Ala-x-peptide is suggested for this peptide. In addition were found a further five peptides which do not occur in normal serum, and two peptides at the site of the Ågren peptide but of a composition differing from that given by Ågren (1949).

METHODS

Amino acids and peptides were isolated on the cation exchanger Zeokarb 225 H^+, washed with water and eluated with 5 n NH_4OH (Prior & Whitehead, 1953). The last solution was evaporated and the remainder dissolved in 10 per cent isopropanol in water. Chromatograms of the isopropanal solution were developed (ascendent) on Whatman No. 1 paper. First run in propanol-water (7 : 3), second run in phenol-water (Ph. 1000 : 277 w., 0.1 per cent cupron) (Boissonnas, 1950). The dried chromatograms were stained by dipping in 0.2 per cent ninhydrin in acetone, and again dried at about 20° C. When an unknown spot was found, one or more chromatograms were made on larger papers and with a larger amount of the test solution. This time the dried chromatograms were stained with a very weak (0.01—0.02 per cent) ninhydrin solution. The unknown spot was cut o eluated with water and this eluate evaporated. T remainder was dissolved in 6 n HCl, placed i tube, which was sealed by melting the open e and hydrolyzed at 110° C for about 12 hours. T tube was then broken, its contents poured on watch glass and evaporated over KOH. The of the HCl was driven off by repeated addit of small amounts of water. The substance was th taken up in 10 per cent isopropanol and chromat graphed. In some cases only a one-dimension chromatogram was made (Dent, 1947).

RESULTS

The composition of the different peptid is given in Table I. The positions of the pe tides in question are shown in Fig. 1, whe all the peptides found are placed in relati to the different amino acids.

The Ala-x-peptide has interested us m since it was found both in the 2 cases of Guglielmo's erythroleukemia and in the cases of myeloic leukemia investigated.

The composition of the peptides was, earlier mentioned, determined by means isolation and acid hydrolysis of the pepti followed by a new chromatographic analy of the hydrolysate. All of the amino ac could not be found in every analysis. T results varied somewhat in different analy Since, however, both the color of the sp and their positions were exactly the same

gnosis	2 cases of erythro-leukemia, 3 cases of myeloic leukemia	2 cases of erythro-leukemia	1 case of erythroleukemia				2 cases of Addisonian anemia
ides	Alanine-x-peptide	Proline y-peptide	Peptide below glycine	Peptide to the left of glutamine	Peptide above glutamine	Peptide giving a fluorescent spot below valine [1]	Peptide at the site of the "Ägren-peptide" [1 2]
he	+	+	+	+	?		+ +
ine				+		+	?
r. ac.	+	+	?	+		+ +	?
m. ac.	+	+		+	?	+ +	+ +
ne	+		+	+		+	+ +
ine.....				+		+	
nes	+			+	+		+
e	+			+		+	?
onine.....	+				+		+
ine							
e				+		+	
e	+		+	+		+	+ +
nine		?		+			
e	+			+	+		+

fter ACTH was given. For further explanation see text.
le isolated from aminosol gave the same spot and the same amino acids.

rent cases it is in all probability a of the same peptides. Since only nounts were available for hydrolysis e easily realized that one or more nts may have been present in too small to be visualized after the n reaction.

Peptides found:

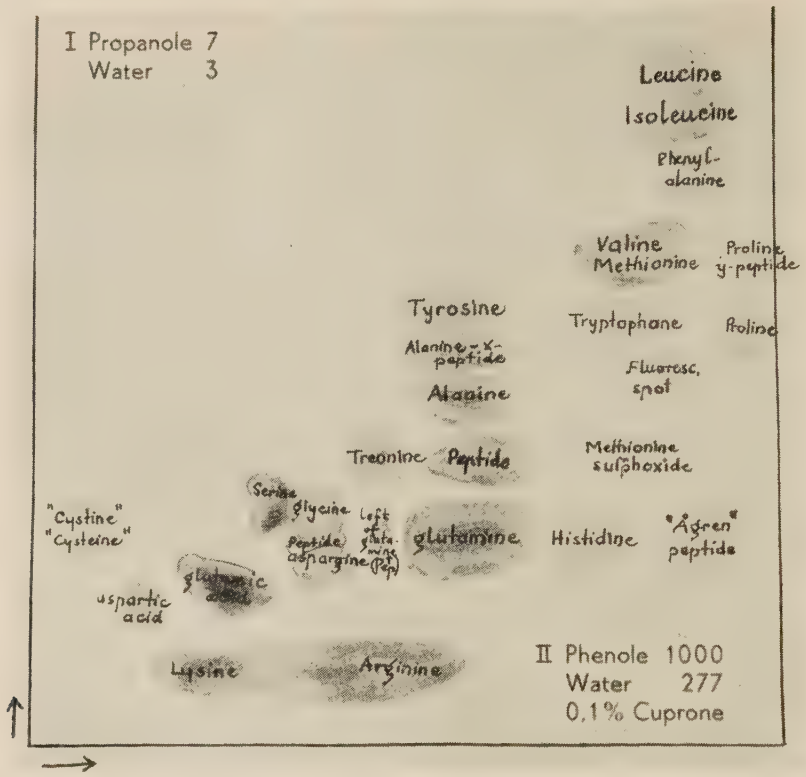
-x-peptide. Found in 2 cases of di o's erythroleukemia and 3 cases of leukemia. If all the amino acids ob- n acid hydrolysis of the substance in the five different cases belong to ptide it would be composed of 10 ids. Alanine was consistently found. this actually belongs to the peptide or accompanies it as a contamination is uncertain.

2. *Pro-y-peptide.* Found in 2 cases of di Guglielmo's erythroleukemia. It gave a small blue spot above proline. After hydrolysis it gave three or possibly four amino acids plus the blue spot.

3. *One peptide below glycine.* Found in one of the cases of erythroleukemia. Content: Alanine, aspartic acid, glycine and serine.

4. *One peptide giving a spot to the left of glutamine.* Found in the same case of erythroleukemia which showed peptide No. 3. After hydrolysis 12 amino acids were found (see Table I).

5. *One peptide giving a large spot above glutamine.* Found in the same case of



erythroleukemia as peptides Nos. 3 and 4. For content see Table I.

6. *A fluorescent spot* was found below valine in the last mentioned patient with erythroleukemia one hour after the administration of 50 mg ACTH. This peptide was composed chiefly of aspartic acid and glutamic acid. On elution from the cation exchanger in the usual manner with 5 n ammonia, only the two amino acids mentioned could be found. If instead we first eluated with n hydrochloric acid and then with n ammonia we found a fluorescent spot in the same place which gave a further 6 amino acids on hydrolysis (see Table I).

7. At the site of the *Ågren peptide* we found a spot in 2 cases of erythroleuke and 2 cases of Addisonian anemia. Only the two latter cases were the peptides furth analysed. According to Ågren (1949) t peptide is composed of hydroxyproline, thionine, lysine and ornithine. In our ca of Addisonian anemia hydrolysis of the p tide gave the following amino acids:

Case 1: Alanine, glutamic acid, glyci serine. A peptide from "aminosol" (a hyd lysate from caseine) gave the same spot the same amino acids.

Case 2: Alanine, glutamic acid, glyci serine, leucines, valine and/or methioni We cannot, however, exclude the prese of arginine, aspartic acid and lysine.

s merely a preliminary report. We aware that an analysis of larger of the respective peptides may lead ding of still more amino acids than ntioned here. At present the patho- ical importance of the peptides re is obscure. Work is continuing in elucidate their importance and to e the amino acid sequence of the

SUMMARY

atographic studies of the amino the serum of 2 cases of di Gugliel- throleukemia revealed a peptide purple spot above alanine. This pep- also found in the serum of 3 cases c leukemia, but could not be found the serum of 50 healthy adults or n of 8 cases of Addisonian anemia. n Ala-x-peptide is suggested for this

ther five peptides were found, but istently, in cases of leukemia. The composition of the peptides was determined by means of isolation and acid hydrolysis, followed by a new chromatographic analysis of the hydrolysate. The positions of the peptides are given in Figure 1, and their composition in Table I. The presence of these peptides may possibly provide a diagnostic clue in doubtful cases. Work is continuing in order to elucidate their patho-physiological importance.

REFERENCES

Boissonas, R. A.: Séparation rapide des acides aminés par chromatographie ascendante bidimensionelle sur papier. Helv. Chim. Acta. *33*, 2, 1966, 1950.

Dent, C. E.: The amino-aciduria in the Fanconi Syndrome. A study making extensive use of techniques based on paper partition chromatography. Biochem. J. *41*, 240, 1947.

Prior, A. P. & Whitehead, T. P.: Paper chromatography of amino-acids in cerebrospinal fluid. Nature *172*, 358, 1953.

Ågren, G.: Paper chromatographic analysis of amino acids and other ninhydrin-reacting substances in deproteinized human plasma. Acta Chem. Scandinav. *3*, 525, 1949.

Scandinav. J. Clin. & Lab. Investigation 46—49, 10, 1958.

SUPRAVITAL NEUTRAL RED STAINING OF LYMPHOCYTI AFTER IRRADIATION

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(Received for publication October 11, 1957)

During some investigations of radiation-exposed persons, Dickie & Hempelman (1947) found that the lymphocytes from these individuals showed an increased number of granules in their cytoplasm. These granules could be stained supravitally with neutral red. Shortly before, by dark field microscopy, Schreck (1947) had shown that after irradiation there was an increase in granules and vacuoles in cyto- and caryoplasm in lymphocytes.

Prigosen (1924) had earlier shown that tumor cells show an increase of neutral red-stainable elements after irradiation, while there is a decrease in the number of mitochondria.

According to the investigation of Gall (1956) and Dickie & Hempelmann (1947) the number of neutral red bodies per cell in the lymphocytes from normal persons will be low; the latter find that less than 20 per cent of the lymphocytes will have over 5 granules per cell. Apart from radiation, unspecific chemical action (Dickie & Hempelmann, 1947) and certain pathological conditions (Lawrence & Todd, 1931) will give rise to an increase in the number of neutral red bodies per cell. Olesen (1936) has investigated the influence of the concentration of the dyestuff on the number of neutral red granules in leukocytes and find in the main, an increase in the number stainable red bodies with increasing concen tration, but this is far from being any co stant phenomenom for the lymphocytes particular.

Gierlach (1936) has shown an increas staining with fluorochromes and neutral r in X-ray damaged cytoplasm in epiderm cells from *Alium cepa*.

The nature of the neutral red stainab bodies has been discussed. McClendon Mitchell (1911—12) thought they belong to the lipoids; on the other hand Harv (1913) considered them to be lecitho-pr teids. More modern authors (Dalton, 195 Dalton & Felix, 1952; Baker, 1951; Thom 1952) call these granules lipochondria definition and some of the authors (Bak 1951; Thomas, 1952) consider that the gr nules take part in the formation of the Gol substance in cells which are prepared acco ing to special techniques.

Recently Hempelmann & Knowl (1953), making some histochemical investig tions on the nature of the neutral red bo found that they can be dissolved in lip solvents. Furthermore they can be stain with fat stains such as Sudan Black B, osm acid and Nile Blue sulphate (blue col

show strong birefrigence and their
e index is between 1.45 and 1.48.
ve negative polysaccharide and nuc-
reactions and the authors believed
icles to consist of phosphate-lipid

ETHODS AND MATERIALS

ml of a saturated alcoholic solution of
d are added 100 ml of absolute alcohol,
olution is spread uniformly over previous
ides, 0.1 ml to each. These are placed
y and dried in warm air so that uniform
utral red are left on the slides. The blood
in a centrifuge tube which contains a
amount of sodium oxalate and is centri-
2—3 minutes (at about 3000 r. p. m.).
y tube is used to take 3—4 drops of the
suspension from the buffycoat, these are
the prepared slide and are spread out to
rcle of about 1 cm diameter. After this
two of the cell suspension is transferred
dry microscope slide (without dye) and
tirred to ensure uniform dyestuff concen-
cover slip with vaselined edges is placed
drop and pressed lightly on the slide, so
ell suspension is protected from the air.
aration is allowed to stand for 1 hour
emperature before it is examined.
ncentration of neutral red used is the
hat recommended by Dickie & Hempel-
47), although the use of Janus green has
ted.
mphocytes are identified by phase contrast
y, and the number of neutral red bodies
s counted by ordinary illumination; 200
es are examined per preparation.
mit between "normal" and "abnormal"
es is quite arbitrarily set at 5 granules
as was done by Dickie & Hempelmann
that lymphocytes with 5 granules and
called "normal" while lymphocytes with
s and more are described as "abnormal".
ntioned before the preparation is allowed
for 1 hour at room temperature before
on. It has been shown that no demon-
anges take place in the preparation after this time, by letting one investigator count the preparation 1 hour after staining and another count it 2—5 hours after staining. No significant alteration in the number of "abnormal" lymphocytes was found. On the other hand after 24 hours almost all the neutral red bodies have disappeared.

The investigations have been carried out on human lymphocytes, and the persons examined can be divided into 3 groups, namely a control group, I, and 2 groups of persons who have been exposed to radiation over a long time, II and III.

Group I comprises unselected persons among the donors from Bispebjerg Hospital's blood bank.

Group II comprises personnel from Finsen Institute and Radiumstation, who work with X-ray diagnosis or X-ray or radiumtherapy; the doses these persons are exposed to vary, but are fixed below 0.3 rem per week.

Group III comprises pers ons with internal contamination after injections of 10—30 ml thorotrast 5—10 years ago. The thorotrast is taken up by the reticuloendothelial tissue and mainly deposited in liver, spleen and bone marrow.

Table I.

"Abnormal" lymphocytes in per cent	Number of cases		
	Group I	Group II	Group III
0— 10	19	0	1
10— 20	15	3	1
20— 30	10	2	1
30— 40	2	7	0
40— 50	2	17	6
50— 60	0	6	6
60— 70	0	8	24
70— 80	0	4	11
80— 90	0	1	4
90—100	0	0	0

RESULTS

Table I shows the distribution of "abnormal" lymphocytes for the 3 groups; it is seen that of 48 cases in the control group, 42 have less than 30 per cent "abnormal" lymphocytes in the blood, while in 48 cases in group II 43 have more than 30 per cent

"abnormal" lymphocytes. The corresponding figures in group III are 54 cases, where 51 have over 30 per cent "abnormal" lymphocytes.

The mean value of abnormal lymphocytes in group III is 62.4 per cent, the corresponding figure for group II is slightly lower, namely 49.8 per cent. In group I, which is the normal group this figure is down to 15.2 per cent. The percentage of lymphocytes with increased number of neutral red bodies was found to be independed of the total number of lymphocytes.

An attempt to investigate the influence of injected dose or dose X time on the percentage of "abnormal" lymphocytes failed to show any significant correlation. In group III there are two cases with less than 25 per cent "abnormal" lymphocytes. The first was a patient with 7.5 per cent "abnormal" lymphocytes, she had a splenectomy 10 years after the injection of thorotrast and 4 years before the present investigation. The other case was a patient with 11 per cent "abnormal" lymphocytes. This patient had an extravascular deposition of thorotrast in the neck, and the injection was made 8 years before the blood-investigation.

The thorotrast dose was not remarkably low in any of these two cases.

It has been shown in the above that irradiation with relatively small doses gives an increase of the neutral red bodies in the lymphocytes; individual investigations with single irradiations with doses down to about 50 r locally also give an increase in the lymphocytes' content of neutral red bodies.

Trowell (1952) has found that lymphocytes from cultured lymph nodes irradiated *in vitro* did not show any change of neutral red stainable vacuoles, or of mitochond- further, Hempelmann & Knowlton (19- failed to find any increase in neutral - bodies in human lymphocytes from he- rinized whole blood irradiated *in vitro* - 1000 r of 250 kv X-rays. A few *in vi-* investigations with irradiation (200 r) human oxalate blood placed in a thermo- at 37° C for one to four hours, failed to sh- any definite increase in the number of neu- red bodies in the lymphocytes. It was - possible to keep the lymphocytes alive - more than about four hours.

Animal experiments showed that lymp- cytes from mice, rats and rabbits norma- show so many neutral red stainable bo- in their cytoplasma, that with the pre- method no changes could be detected a- irradiation.

SUMMARY

Continuous irradiation with small d- gives a greater percentage of lymphocy- with increased numbers of neutral red sta- able bodies in the cytoplasma.

ACKNOWLEDGEMENT

I wish to thank Dr. A. Fløjstrup and Dr. Therkildsen for advice and help with the exp- ments.

REFERENCES

Baker, J. R.: The Golgi substance. Nature *1* 1089, 1951.

Dalton, A. J.: Observations of the Golgi substa- with the electron microscopi. Nature *168*, - 1951.

Dalton, A. J. & Felix, M. D.: Lipochondria a the Golgi substance in the epithelial cell the epididymis. Nature *170*, 541, 1952.

Dickie, A. & Hempelman, L. H.: Morpholo- changes in the lymphocytes of persons exp- to ionizing radiation. J. Lab. & Clin. M *53*, 1045, 1947.

A.: A previously underscribed granlue
n the lymphocyte. Am. J. Med. Sci. *191,*
1936.
J. S.: Symposium of Radiobiology. Ed.
son J. Publ. Wiliy, London, 1952, pp.
392.
. N.: A critisism on the indicator methods
terminicy cell permeability for alkalies.
J. Physiol. *31,* 335, 1913.
ann, L. H. & Knowlton, N. P.: Nature
utral red staining refractive particles in
nocytes of blood of normal humans. Blood
, 1953.
J. S. & Todd, H.: Variations in charac-
ics of lymphocytes in supravital prepara-
Fol. Haematol. *18,* 318, 1931.
n, J. F. & Mitchell, F. H.: How do iso-
tonic chloride solution and other partenogenetic agents increase oxidation in the sea urchin egg? J. Biol. Chem. *10,* 459, 1911—12.
Olesen, M.: Nogle undersøgelser over supravitalfarvning i saltvand. Thesis. Munksgaard, Copenhagen, 1936, p. 21, 44.
Prigosen, R. E.: Vital staining of tumor cells after roentgen rays. J. Cancer *8,* 305, 1924.
Schreck, R.: Dark field observations on lymphocytes exposed to X-rays and other injurians agents. Proc. Soc. Exper. Med. Biol. *64,* 381, 1947.
Thomas, O. L.: Lipochondria of living nerve cells. Science *115,* 657, 1952.
Trowell, O. A.: The sensitivity of lymphocytes to ionizing radiation. J. Path. & Bact. *64,* 187, 1952.

Scandinav. J. Clin. & Lab. Investigation 50—52, 10, 1958.

DETERMINATION OF PROTEIN IN CEREBROSPINAL FLUID

A COMMENT ON THE LOWRY METHOD

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(Received for publication October 12, 1957)

On the basis of the color reaction with Folin's phenol reagent, Lowry, Rosebrough, Farr & Randall (1951) have proposed a very sensitive and simple method for the determination of protein. Daughaday, Lowry, Rosebrough & Fields (1952) have reported that the method is suitable for the determination of protein content of cerebrospinal fluid (c. s. f.) and Lous, Plum & Schou (1956) have recommended the method for routine determinations.

In this laboratory, however, the method invariably has given higher values than determinations with other methods. It was suspected that the cause of this discrepancy consisted in a varying content of color producing non-protein substances (such as tyrosine, tryptophan, acetic salicylic acid, phenacetine, chloropromazine *etc.*) in the different c. s. f. samples. Daughaday *et al.* (1952) report an average correction value due to these color producing non-protein substances of 0.06 mg/ml and recommend that this value be subtracted from the protein values determined. It is, however, unlikely that the amount of color producing non-protein substances is constant from sample to sample. Therefore, the actual amounts of these substances have been determined in c. s. f. samples from neurosurgical neurological and psychiatric patients. Furthermore, it was examined whether the accuracy of the Lowry method could be increased by individual correction values.

METHODS

The *protein determination* was carried out according to the instructions given by Daughaday *et al.* (1952) using a tyrosine solution (0.13 mg% in 0.1 N hydrochloric acid as a standard. This standard represents a protein concentration of 0.1 mg/ml. A Beckman spectrophotometer model DU was used; the extinctions were read at 700 mμ.

The amount of color producing non-protein substances (the *correction value*) was determined by analysing a deproteinized extract of the c. s. f. sample.

Deproteinization: In a small centrifuge tube (5 · 50 mm) 0.05 ml of perchloric acid (60 per cent, sp. gr.: 1.54) was added to 0.5 ml of a c. s. f. sample. After careful mixing and standing for 5—15 minutes at room temperature the tube was centrifuged for 10 minutes at about 6000 g. Duplicate samples of 0.2 ml of the supernatant were taken for analysis.

Calculation: The correction value was calculated as

$$\frac{E_c \cdot 1.1 - E_b}{E_s - E_b} \cdot 0.5 \text{ mg/ml}$$

The protein concentration calculated according to Daughaday *et al.* (1952) with a fixed correction value was

$$\frac{E_p - E_b}{E_s - E_b} \cdot 0.5 - 0.06 \text{ mg/ml}$$

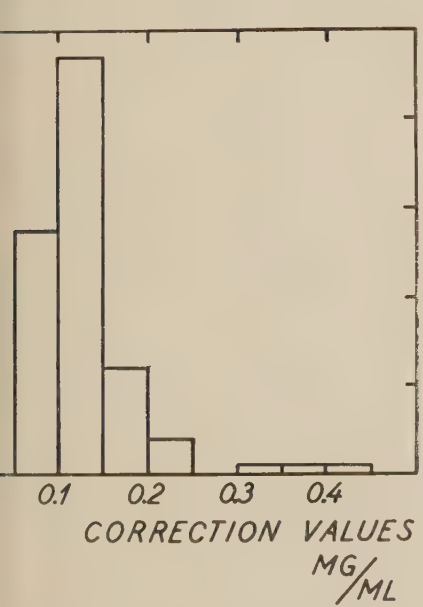
in concentration calculated with individual values was

$$\frac{E_p - E_e \cdot 1.1}{E_s - E_b} \cdot 0.5 \text{ mg/ml}$$

E_s and E_p are the extinctions for blank, acid extract, standard and sample respec- ne factor 1.1 was introduced in order to r the dilution of the sample by the addition oric acid.

RESULTS

ears from Figure 1 that c. s. f. samp- ined most varying amounts of color g non-protein substances corre- to correction values ranging from .45 mg/ml. In the material presented use of a fixed correction value of /ml — as recommended by Daugha- l. (1952) and Lous *et al.* (1956) — ntroduce errors in the protein deter- is ranging from 0 to 0.39 mg/ml. As



Distribution of correction values from de- ons on 94 cerebrospinal fluid samples from gical, neurological and psychiatric patients.

Table I. *Protein determinations with Lowry's method and a tensiometric method.*

Lowry's method with fixed corrections mg/ml	Lowry's method with individual corrections mg/ml	Tensiometric determinations [1] mg/ml
0.41	0.31	0.29
0.40	0.30	0.31
0.35	0.27	0.25
0.55	0.41	0.39
0.43	0.31	0.30
0.37	0.28	0.28
0.30	0.26	0.25
0.09	0.08	0.07
0.77	0.68	0.67

[1] The tensiometric determinations were carried out by Dr. L. Uhrbrand.

compared to the normal range of protein concentrations in human c. s. f. samples (0.1—0.4 mg/ml) such errors are considerable.

In order to decide whether individual correction values would increase the accuracy of the Lowry method, such determinations were compared with protein values determined by a tensiometric method whose accuracy has been controlled with micro Kjeldahl determinations (Uhrbrand, 1956). The figures of Table I show that the individually corrected values agree with the tensiometric determinations while no such agreement was found when a constant correction was used. To avoid large errors in the protein determination it is therefore recommended that a perchloric acid extract be analyzed with the sample as described above.

SUMMARY

Protein determination in cerebrospinal fluid according to Lowry's method involves a fixed correction for color producing non-

protein substances. The actual content of color producing non-protein substances in c. s. f. samples from 94 hospitalized patients varied considerably and it is recommended to include individual correction values in the determination.

REFERENCES

Daughaday, W. H., Lowry, O. H., Rosebrough, H. J. & Fields, W. S.: Determination of cerebrospinal fluid protein with the F phenol reagent. J. Lab. & Clin. Med. *39*, 1952.

Lous, P., Plum, C. M. & Schou, M.: Kolorime bestemmelse af totalprotein og globuli spinalvædske. Nord. Med. *55*, 693, 1956.

Lowry, O. H., Rosebrough, N. J., Farr, A. L Randall, R. J.: Protein measurement with Folin phenol reagent. J. Biol. Chem. *193*, 1951.

Uhrbrand, L.: Personal communication. 1956

. J. Clin. & Lab. Investigation 53—58, 10, 1958.

JORIMETRIC METHOD FOR MEASURING THE ACTIVITY IN SERUM OF THE ENZYME GLUTAMIC PYRUVIC TRANSAMINASE

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(Received for publication October 24, 1957)

nzyme glutamic pyruvic transaminase s the following reaction:

ine + α-ketoglutarate ⇌ pyruvate + te.

nzyme activity can be expressed as unt of pyruvate produced at a certain The amount of pyruvate can be ed by adding lactic dehydrogenase duced diphosphopyridine nucleotide H). Pyruvate is transformed to lac- DPNH to diphosphopyridine nuc- (DPN). The increase in the amount equals the amount of pyruvate con- o lactate.

method for determining the enzyme pyruvic transaminase which is to be ed in this paper is based on a fluori- determination of diphospyridine nuc- (DPN).

has no native fluorescence but it can fluorescent by suitable reagents. The of acetone to DPN forms a con- n product which yields a high fluor- This method of determining DPN m has been introduced by Levitas, n, Rosen, Huff & Perlzweig (1947). er & Kodicek (1950) and later Burch, Storvick, Bicknell, Kung, Alejo, Everhart, Lowry, King & Bessey (1955) have, instead of acetone, used methyl ethyl ketone for developing the fluorescence. Methyl ethyl ketone is less volatile than acetone and the condensation product gives twice as much fluorescence. Greengard (1956) has used this principle in estimating pyruvate. In the presence of lactic dehydrogenase, and reduced diphosphopyridine nucleotide (DPNH) pyruvate is transformed to lactate, and equimolar amounts of DPNH are transformed to DPN. DPN is measured by condensation with methyl ethyl ketone in an alkaline medium. In this way pyruvate can be determined in amounts of $2.5 \cdot 10^{-11}$ mole. Lowry, Roberts & Kapphahn (1956) have used a similar procedure for estimating the enzymes lactic dehydrogenase and 6 — phosphogluconic dehydrogenase in tissue. In this procedure DPN is treated with alkali and not with methyl ethyl ketone.

In the method described in this paper the fluorescence of DPN is developed by condensation with methyl ethyl ketone. The principle of the method can be used for the determination of the activity in serum of other enzymes if the determination depends on a change of DPNH to DPN. So far we have developed fluorimetric procedures (to

d by a grant from Eli Lilly and Company, olis.

be published) for determining the activity in serum of glutamic oxalacetic transaminase and lactic dehydrogenase.

METHOD

Reagents.

a) Phosphate buffer, M/15, pH 7.4.

b) α-ketoglutarate M/10, dissolved in buffer (a). pH is adjusted to 7.4.

c) l-alanine, 1.78 gm of l-alanine and 1.30 gm of anhydrous dibasic potassium phosphate are dissolved in water, pH is adjusted to 7.4 and the solution is diluted to 100 ml with water.

d) Nicotinamide, 1 gm/100 ml, dissolved in buffer (a).

e) DPNH (Boehringer & Sohne), 6 mg/ml dissolved in M/10 Tris buffer, pH 8.0.

f) Lactic acid dehydrogenase (Boehringer & Sohne, crystal suspension, about 5 mg enzyme protein per ml). Diluted 1 to 40 with buffer (a), daily prepared.

g) Trichloroacetic acid, 10 gm/100 ml.

h) Methyl ethyl ketone.
20 μl manganese chloride solution ($MnCl_2$, 4 H_2O, 1 gm/100 ml) and 10 ml methyl ethyl ketone are mixed, daily prepared.

i) Potassium hydroxide, 5 N.

k) Hydrochloric acid, 2 N.

l) Monobasic potassium phosphate, 20 gm/100 ml.

m) Quinine sulfate, 0.2 mg/100 ml, dissolved in 0.6 N sulfuric acid.

n) DPN (Boehringer & Sohne, preparation "reinst").
The solutions a, b, c, d, e, and f are stored at 5° C.

Apparatus.

Different sizes of constriction pipettes and syringe pipettes. Matched test tubes for fluorimetric measuring.

Fluorometer (Kipp)

Primary filter	max. transmission	360 mμ.
Secondary filter	max. transmission	480 mμ.

Procedure

Determination of the sample

0.2 ml serum, 0.3 ml nicotinamide (d), 0.05 DPNH (e), and 0.05 ml lactic acid dehydroge (f) are mixed in a 10 ml centrifuge tube with ro bottom. The tube is placed in a water bath at centigrade for about 10 min. 0.7 ml substrate w is made up of 5 parts of l-alanine (c) and 2 pa of α-ketoglutarate (b) is added, and the time no on a stop watch. After exactly 10. min. 0.5 ml chloroacetic acid (g) is added to inactive the tra minase. After centrifugation 0.5 ml of the sup natant is pipetted into a matched test tube 0.5 ml methyl ethyl ketone (h) is added. Af shaking 0.2 ml 5 N Potassium hydroxide (i added, the tube is again well shaken, 0.8 ml 2 hydrochloric acid (h) is added and the tube again shaken. After 5 min. at room temperat the tube is placed in a boiling water bath for min. to remove the methyl ethyl ketone. Fina 0.5 ml potassium phosphate (1) and 6 ml wat are added and the grade of fluorescence of t solution is read in a fluorometer. A 0.2 mg p cent solution of quinine sulfate (m) is used as fluorometer standard. This standard should give fluorometer reading of 100.

Determination of the blank

The mixture of serum, DPNH, lactic acid hydrogenase, and nicotinamid is placed in a wa bath at 37° centigrade for about 10 min. 0.5 trichloroacetic acid is added, and followed by 0.7 of the substrate. The same procedure as for sample determination is then followed.

Determination of spontaneous transaminat

This determination is done exactly as descri for the serum determination (including sample blank), except that 0.2 ml serum is substituted 0.2 ml buffer.

This determination is made daily.

Calculation

A DPN standard curve is prepared by ad DPN (n) solutions in different concentration the reaction mixture instead of serum. No DP

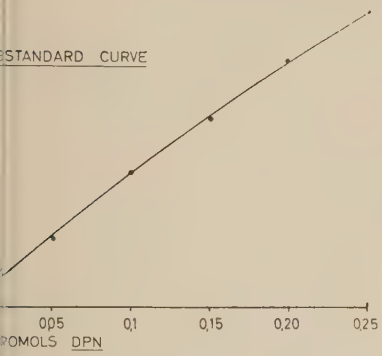

Fig. 1. DPN standard curve.

The procedure for the serum blank deter- is then followed. DPN is added in om 0 to 0.25 micromols. Fig. 1 shows the urve.

he calibration curve is found the number ols DPN in sample solution. A, and in solution, B.

lues for the determination of the spon- ansamination are found in the same way mple, C, and for the blank, D.

mber of micromols DPN produced per m per 10 minutes is: (A—B) — (C—D). t equals the number of micromols pyru- ced.

zyme activity is expressed as the number nols pyruvate produced per hour per

ESULTS AND DISCUSSION

ariation in the concentration of the substrate:

fluence of the concentration of the (l-alanine and α-ketoglutarate) investigated. Each part of the sub- s been tested separately. Fig. 2 and ow the relation of the enzyme acti- the concentration of l-alanine and tarate respectively.

of l-alanine (c) and 0.2 ml of

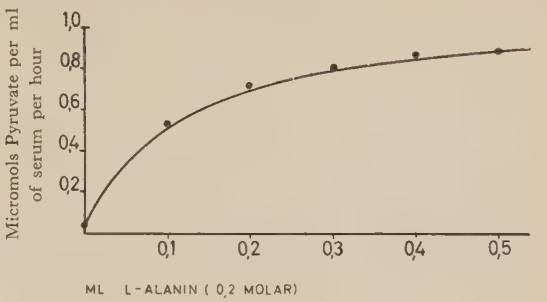

Fig. 2. The enzyme activity as a function of the concentration of l-alanine.

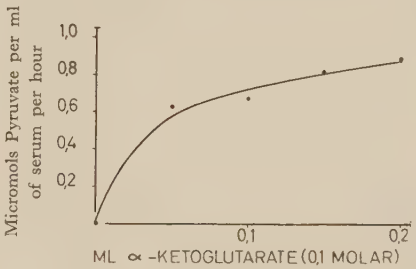

Fig. 3. The enzyme activity as a function of the concentration of α-ketoglutarate.

α-ketoglutarate (b) have been used for routine analysis.

It has been shown by 34 comparative experiments that dl-alanine in twice as strong a concentration as l-alanine can be used instead of l-alanine.

DPNH is added in surplus and the concentration used was found to have no influence on the enzyme activity.

The serum blank.

The value for the serum blank determination includes the value for the preformed pyruvate. For this determination it has been found that it does not make any difference whether the mixture of serum, nicotinamide, DPNH, and lactic acid dehydrogenase has

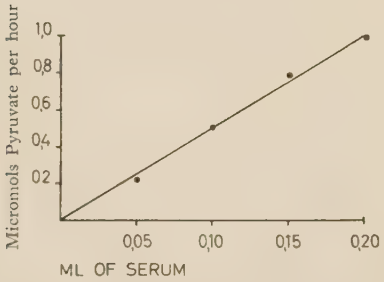

Fig. 4. The enzyme activity as a function of the amount of serum.

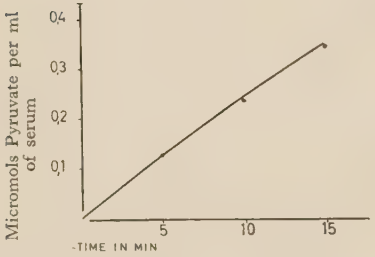

Fig. 5. The enzyme activity as a function of time.

been standing 5, 10, or 20 minutes at 37° centigrade before trichloroacetic acid is added. This means that the oxidation of DPNH after a 5 minute period does not proceed in a measurable degree unless l-alanine and α-ketoglutarate are added.

The relation of the enzyme activity to the amount of serum, the incubation time, the temperature, and the pH

Fig. 4 shows the relation of the activity to the amount of serum of which 50 μl to 200 μl have been added. It is seen that the enzyme activity is almost proportional to the amount of serum added. It should be mentioned here that if the enzyme activity on

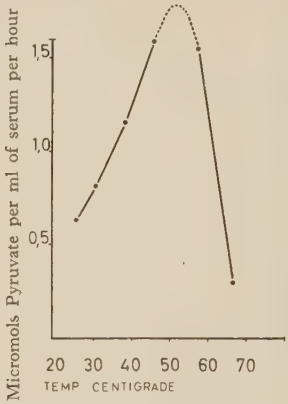

Fig. 6. The enzyme activity as a function of temperature.

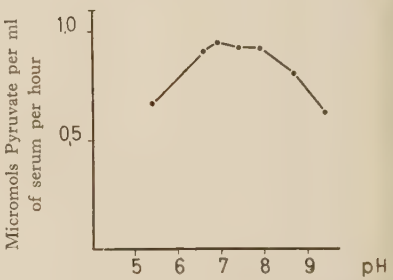

Fig. 7. The enzyme activity as a function of

addition of 200 μl serum is so high that reading on the fluorometer is over 100, test should be done again with 100 μl seru

Fig. 5 shows the influence of the incu tion time on the enzyme activity. A 10 nute incubation time is used for the rout analysis.

Fig. 6 shows the influence of the tem rature on the enzyme activity. This exp ment has been performed with a 10 min incubation time. The Q_{10} value calculated the interval from 30° to 40° centigrade found to be 1.6. The importance of keep a constant temperature during the incuba is obvious.

shows the influence of pH on the ctivity. It is seen that the enzyme ptimum pH about 7; pH 7.4 has for the routine analysis.

evelopment of the fluorescence

ndensation product of DPN and hyl ketone has a high fluorescence, stable for at least 24 hours.
has a native fluorescence which ere with the fluorimetric measuring This native fluorescence of DPNH ed by acidification (Lowry *et al.*,

ible decomposition of DPN by the PN-ase is avoided by adding nico- (McIlwain & Rodnight, 1949).
ne and α-ketoglutarate have no in- the development of the condensa- luct of DPN and methyl ethyl

Recovery experiments

ry experiments with pyruvate have at this substance can be recovered 97 per cent and 103 per cent.

Accuracy

ndard deviation on the mean value plicates, calculated from 98 dupli- 0.1 unit.

Normal values

termination has been done on serum healthy blood donors. A mean value ts with a standard deviation of 0.5 found.

Adaptation of the method to smaller amounts of serum

It should be mentioned that the method can easily be adapted to the use of 100 μl serum instead of 200 μl serum. This can be done by taking half the amount of substrate, trichloroacetic acid and serum. It will still be possible to get 0.5 ml of the supernatant for developing the condensation product. Another possibility is to increase the sensitivity of the fluorometer. In that way smaller amounts of supernatant can be used, which makes it possible to use still smaller amounts of serum.

Comparison with other methods:

The method described has been compared with a colorometric method which involves an estimation of the produced pyruvate by adding dinitro-phenylhydrazin to form pyruvate-dinitro-phenylhydrazone (Wroblewski & Cabaud, 1957).

Fig. 8 illustrates the comparison.

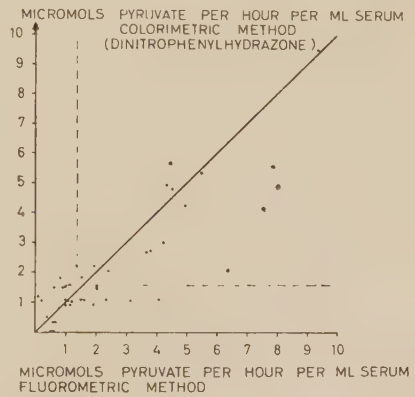

Fig. 8. The comparison between the fluorimetric method and the colorimetric method of Wroblewski & Cabaud. The dotted line indicates the upper limit for the normal values.

40 different sera have been tested with both methods. It is seen that there is a rather big discrepancy. No satisfactory explanation has been found for this phenomenon, but it can be stated that experiments with the colorimetric method have shown that only 87 per cent of the pyruvate can be recovered in some cases. Furthermore, no constant relation between the enzyme activity and the amount of serum added has been found when using the colorimetric method of Wroblewski & Cabaud.

SUMMARY

A method for the determination of glutamic pyruvic transaminase (GPT) in serum is presented.

The procedure runs in two steps.

I: Serum is incubated 10 minutes at 37° C in a substrate made up of l-alanine, α-ketoglutarate, nicotinamide, DPNH, and lactic dehydrogenase.

II: DPN formed is measured by the fluorescence of its ketone condensation product.

The method is found to be sensitive, reproduceable and well suited as a routine method for a hospital laboratory.

REFERENCES

Burch, H. B., Storvick, C. A., Bicknell, L., Ku... H. C., Alejo, L. G., Everhart, W. A., Low... O. H., King, C. C., & Bessey, O. A.: M... bolic studies of precursors of pyridine nuc... tide. J. Biol. Chem. *212,* 897, 1955.

Carpenter, K. J., & Kodicek, E.: The fluorim... estimation of N¹-Methylnicotinamide and ... differentiation from coenzyme I. Biochem ... *46,* 421, 1950.

Greengard, P.: Determination of intermedi... metabolites by enzymic fluorimetry. Na... *178,* 632, 1956.

Levitas, N., Robinson, J., Rosen, F., Huff, J. ... & Perlzweig, W. A.: The fluorescent c... densation product of N¹-Methylnicotinamid... Acetone. J. Biol. Chem. *167,* 169, 1947.

Lowry, O. H., Roberts, N. R., & Kapphahn, J. ... The fluorometric measurement of pyrid... nucleotides. J. Biol. Chem. *224,* 1047, 1957.

McIlwain, H. & Rodnight, R.: Breakdown ... cozymase by a system from nervous tis... Biochem. J. *44,* 470, 1949.

Wroblewski, F. & Cabaud, P.: Colorimetric M... surement of serum glutamic pyruvic tra... minase. Am. J. Clin. Path. *27,* 235, 1957.

J. Clin. & Lab. Investigation 59—62, 10, 1958.

)-DIMENSIONAL PAPER CHROMATOGRAPHIC METHOD FOR THE SEPARATION OF BARBITURATES

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(Received for publication September 4, 1957)

ost common methods for the quan-
stimation of barbiturates are based
fact that barbiturates in alkaline
ave a strong ultra-violet absorption
e, 1940; Goldbaum, 1952; Lous,
hese spectrophotometric methods
information as to what particular
e is analysed, though it is possible
ome conclusions from the solubility
xact wave-length of the absorption
. The chromatographic analysis,
s of greatest value in this respect.
one-dimensional paper chromato-
methods have been suggested, dif-
the choice of the developing sol-
d the way of detecting the barbi-
n the chromatograms. Algieri &
1952) and Riebeling & Burmeister
se *e. g.* n-butanol saturated with
. Sabatino (1954) prefers ethylene
whereas Allgén (1953, 1957) re-
od results with iso-amyl alcohol
with ammonia in ammonia atmos-
übner & Pfeil (1954) recommend
ohol and butanol saturated with
. The best separation so far seems,
to have been achieved with diffe-
tures of chloroform (Wickström &
1952; Wright, 1954; Deininger,

Some of the investigators detect the barbiturates after spraying the chromatograms with more or less specific reagents (Sabatino, 1954; Hübner & Pfeil, 1954; Wickström & Salvesen, 1952; Deininger, 1955) while Grieg (1952), Riebeling & Burmeister (1954), Allgén (1953, 1957) and Wright (1954) locate the barbiturates in alkaline environment as dark spots in ultra-violet light.

As it hardly seems possible to separate the multitude of barbiturates with sufficient accuracy even with these above-mentioned one-dimensional methods, this investigation was carried out in an attempt to elaborate a two-dimensional paper chromatographic method for the separation of barbiturates.

MATERIAL

The barbiturates analysed in this investigation are listed in the table. As the method was intended chiefly for use in cases of barbiturate intoxication such as suicidal attempts, drug addiction *etc.*, only babiturates for oral intake were included.

METHODS

Reagents

Amyl acetate A. R. Chloroform purissum stabilized with 1 per cent ethanol. Concentrated ammonia A. R.

Procedure

10 µl of a chloroform solution of barbiturate is applied with a micropipette to a Whatman 1 chromatographic sheet, 48 × 54 cm, which is then allowed to equilibrate for at least one hour in a tank saturated with chloroform and ammonia. The chromatogram is developed at room temperature by descending technique with the chloroform phase of a mixture of two parts of chloroform and one part of concentrated ammonia (v/v).

After drying at room temperature for about five minutes the paper is suspended in another tank saturated with amyl acetate and ammonia and allowed to equilibrate for at least one hour. The chromatogram is then run at right angles to the first direction by descending technique with the amyl acetate phase of a mixture of two parts of amyl acetate and one part of concentrated ammonia (v/v). It is necessary to saturate the chromatographic tanks with fresh concentrated ammonia immediately before the run.

After being dried at 100° C for five minutes chromatograms are treated with ammonia vapo five minutes and examined in ultra-violet l from a mercury lamp with maximal emissio about 2600 Å (type LUS, manufactured by LU Stockholm). The barbiturates appear as dark l spots on a light blue background. The effect be considerably accentuated by putting a fluores screen between the chromatogram and the obser (Grieg, 1952). The screen might *e. g.* be a qu plate coated with a thin film of cadmium bo suspension (the suspension is supplied by LU Stockholm).

The complete procedure will take about sev hours. By this method it is possible to detect ba turates in amounts from 10 to 40 µg.

An additional possibility to distinguish betw the different barbiturates is offered after spray the chromatogram with a 0.02 M potassium perm ganate solution. The barbiturates containing reducing radical in their molecule can then i

Table. *Rf-values of barbiturates after two-dimensional paper chromatography. (NFN indicates name accepted by Nordiska Farmakopénämnden.)*

Number	Name	Chemical structure	Rf in second solvent: amyl acetate/ammonia	Rf in first solvent: Chloroform/ ammonia	Reduction of
1	allypropymal (NFN)	5-isopropyl-5-allyl barbituric acid	.14	.20	+
2	diallymal (NFN)	5,5-diallyl barbituric acid	.06	.10	+
3	diemal (NFN)	5,5-diethyl barbituric acid	.03	.04	–
4	diminal	5-ethyl-5-(1-methyl-butenyl) barbituric acid	.18	.15	+
5	eldoral	5-ethyl-5-piperidyl barbituric acid	.04	.04	–
6	enhexymal (NFN)	5-methyl-5-cyclohexenyl-N-methyl barbituric acid	.75	.58	(+
7	enphenemal (NFN)	5-ethyl-5-phenyl-N-methyl barbituric acid	.74	.50	–
8	gemonit	5,5-diethyl-N-methyl barbituric acid	.62	.40	–
9	hexemal (NFN)	5-ethyl-5-cyclohexenyl barbituric acid	.15	.14	(+
10	ibomal (NFN)	5-isopropyl-5-(ß-brom-allyl) barbituric acid	.12	.17	+
11	idobutal	5-n-butyl-5-allyl barbituric acid	.40	.39	+
12	mebumal (NFN)	5-ethyl-5-(1-methyl-butyl) barbituric acid	.52	.57	–
13	medapan	5-ethyl-5-cycloheptenyl barbituric acid	.30	.24	–
14	pentymal (NFN)	5-ethyl-5-isoamyl barbituric acid	.55	.51	+
15	phenallymal	5-allyl-5-phenyl barbituric acid	.05	.09	+
16	phenemal (NFN)	5-ethyl-5-phenyl barbituric acid	.03	.09	–
17	soneryl	5-ethyl-5-butyl barbituric acid	.30	.33	(+
18	vesperon	5-allyl-5-(ß-brom-allyl) barbituric acid	.06	.11	–

be seen as yellow spots on a red back- hereas the derivatives with a cyclo- r cycloheptenyl group show a delayed and appear as yellow spots about a er the spraying.

RESULTS

-values for the eighteen barbiturates o solvent systems are listed in the ch pair of Rf-values is the mean of endent estimations. The positions of urates after two-dimensional paper graphy are visualised in the Figure, e reducing property of the barbitu- narked as well.

soon realised that the Rf-values be relied upon for the identification wn spots. It was therefore necessary ses to run a test solution under the ditions for comparison.

DISCUSSION

s two-dimensional paper chromato- method it might be possible to dis- between at least thirteen barbituric vatives out of eighteen. This method this respect superior to the two- al procedure suggested by Romano who reported Rf-values between .0. It is interesting to note that the graphically fastest moving barbi- ostly belong to the short-acting ones the slow-moving are long-acting, of great value to know in clinical ntoxication. The N-methylated bar- have considerably higher Rf-values non-methylated ones.

SUMMARY

-dimensional paper chromatographic is described using chloroform-am-

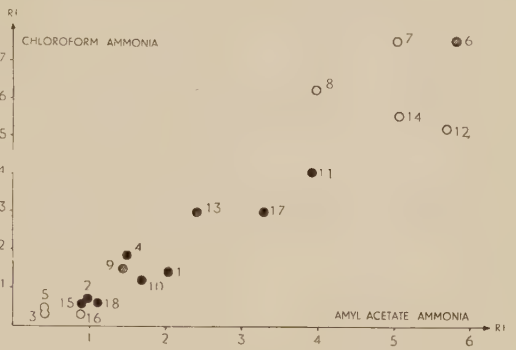

Fig. *Two-dimensional paper chromatography of barbiturates.* The barbiturates are represented by numbers according to Table. ● signifies immediate reduction, ▨ delayed reduction and O no reduction of potassium permanganate.

monia and amyl acetate-ammonia systems for the separation of eigtheen barbituric acid derivatives.

REFERENCES

Algieri, E. J. & Walker, J. T.: Paper chromatography for identification of the common barbiturates. Am. J. Clin. Path. *22*, 37, 1952.

Allgén, L.-G.: Paper chromatographic identification of barbiturates. Sv. Farm. Tidskr. *57*, 188, 1953.

Allgén, L.-G.: Separation and identification of barbiturates in biological material by paper chromatography, Scandinav. J. Clin. Lab. Investigation *9*, 71, 1957.

Deininger, R.: Papierchromatographischer Nachweis der Barbitursäuren. Arzneimittelforsch. *5*, 472, 1955.

Eldwidge, W. F.: Absorption spectrophotometry in pharmacological analysis II. Quart. J. Pharm. & Pharmacol. *13*, 219, 1940.

Goldbaum, L. R.: Determination of barbiturates. Ultra-violet spectrophotometric method with differentiation of several barbiturates. Anal. Chem. *24*, 1604, 1952.

Grieg, A.: Paper chromatographic separation of barbituric acids. Nature *170*, 845, 1952.

Hübner, G. & Pfeil, E.: Detection of barbiturates. Hoppe-Seyler's Ztschr. phys. Chem. *296*, 225, 1954.

Lous, P.: A spectrophotometric method for determining barbituric acids in body fluids. Acta pharmacol. et toxicol. *10*, 134, 1954.

Riebeling, C. & Burmeister, H.: Papierchromatographischer Nachweis kleinster Barbitursäuremengen. Klin. Wchnschr. *32*, 1057, 1954.

Romano, C.: Nuove tecniche per l'estrazione e l'identificazione di alcuni veleni: alcaloidi, barbiturici, glucosidi. Arch. Sc. Biol. Bologna *36*, 543, 1952.

Sabatino, F.: The isolation and identification of barbiturates. J. Ass. Off. Agr. Chem. *37*, 1954.

Wickström, A. & Salvesen, B.: The separation identification of some barbiturates by p chromatography. J. Pharm. & Pharmac 98, 1952.

Wright, J. T.: A rapid method of identifying p cular barbiturate derivatives in small sam of blood by paper chromatography. J. Path. *7*, 61, 1954.

. J. Clin. & Lab. Investigation 63—66, 10, 1958.

NT IRON DEFICIENCY AFTER REPEATED REMOVAL OF BLOOD IN BLOOD DONORS

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(Received for publication October 25, 1957)

y humans maintain an adequate iron by a very narrow margin (Moore, After a large iron depletion, such as . *g.* in blood donors, the organism ly slowly to substitute from the food askins, Stevens, Finch & Finch, A manifest iron deficiency anemia s develop in some of the more fre- lood donors (see *e. g.* Sköld & tröm, 1940; Laurell, 1947; Remy, & Krüger, 1952; Möller, 1952; olländer, Undritz & Zehnder, 1955; & Ramgren, 1956).

ver, Kaldor (1953) found that the of any significant change in the level iron in his material suggested that tabolism is readily adapted to in- demands. Likewise, Davis (1953) rum iron values within the accepted f normality in blood donors, in- the absence of iron deficiency. The ncy in the findings referred to above due to differences in nutritional and in the amount of blood that oved per time unit in the different s. As these have not generally been according to the frequency of the tings, it is not possible to draw any nclusions as to how often the average nor can be bled without provoking any untoward signs of a negative iron balance. A common, more or less empirical schedule has been to recommend intervals of 2—3 months, and in some places still shorter intervals are used.

An early biochemical symptom indicating a negative iron balance in non-anemic individuals has been pointed out by Laurell (1947). He found that a tendency toward lowering of the serum iron usually is accompanied by an increase of the total iron-binding capacity (TIBC) of serum, resulting in a low saturation index (*i. e.* the serum iron as a percentage of the TIBC).

The purpose of this investigation has been to study these parameters in a sufficiently large number of blood donors, grouped according to the relative frequency of blood-lettings during the preceding year, in order to be able to recommend a safe schedule for the blood donor services.

MATERIAL AND METHODS

The material consisted of 136 presumably healthy male blood donors picked out at random at the Blood Donor Service of the University Hospital of Uppsala. Their median age was 26 years with a range of 18 to 49 years. Only those with a hemoglobin concentration of more than 12.3 g per 100 ml were accepted as donors and included in the material. The blood samples were drawn im-

mediately before the donors gave their 350 ml-contribution to the blood bank. All samples were drawn between 9 and 10 a. m., and the donors were in the postabsorptive state. The history of their previous donations during the last years was noted from the records.

The material was divided in four groups, of which group I consisted of the normals, *i. e.* those who were bled for the first time or who had not been bled during the last 12 months, group II of those who had given 1—2 units of 350 ml, group III 3—4 units, group IV 5 units or more of blood during the last 12 months. The intervals were not quite regular but the interval between the preceding blood letting and the drawing of the blood sample was in no case less than one month, and in most cases two months or more. None of the donors had received supplementary iron during the last 12 months.

Serum iron was determined by the method of Vahlquist (1941) as modified by Hagberg (1953). The unsaturated iron-binding capacity of serum was estimated according to Rath & Finch (1949). The total iron-binding capacity (TIBC) was obtained by adding the values of the serum iron and the unsaturated iron-binding capacity. For evaluation of the methods, see Hagberg (1953). Hemoglobin was determined spectrophotometrically as oxihemoglobin. The serum protein concentrations were estimated gravimetrically by van Slyke's $CuSO_4$ method.

The material was treated statistically accord to conventional methods. Differences between n values exceeding thrice their standard error h been regarded as statistically significant, and di ferences between 2½ and 3 times their stand error as statistically very probable.

RESULTS AND DISCUSSION

The results are shown in Table I. T serum iron, which in the control group h a value of 125 micrograms per 100 m showed a tendency to rise during the fir four donations, reaching a maximum of 14 a rise that was found to be statistically sign ficant and that might perhaps be interpret as a sign of an activation of the iron tra port. When five units of 350 ml or ma had been removed per year the serum ir declined to 108 micrograms per 100 ml. figure still within the normal range but we below that of the control group and sign ficantly lower than the maximum value group III.

The TIBC demonstrated a slow increa with increasing frequency of blood donation ending up in the last group with a value 407 micrograms per 100 ml, the differen

Table I. *Serum iron, total iron-binding capacity, saturation index, hemoglobin and total serum protein in blood donors*

Group	Number of donors	Number of 350 ml-units bled during preceding 12 months	Serum iron microg per 100 ml			TIBC microg per 100 ml			(SI/TIBC) × 100 per cent			Hemoglobin g/100 ml	Serum protein
			M	e(M)	SD	M	e(M)	SD	M	e(M)	SD		
I	23	0	125.0	5.77	27.7	360.2	11.9	57.0	35.6	1.91	9.16	14.9	
II	39	1—2	144.9	5.49	34.3	360.4	7.83	48.9	41.1	1.59	9.91	15.0	
III	48	3—4	148.1	4.49	31.1	388.1	8.35	57.7	39.6	1.68	11.61	14.9	
IV	26	5 or more	107.7	6.26	31.9	406.9	11.2	57.3	27.3	1.97	10.04	14.7	

M = mean value
e(M) = standard error of the mean
SD = standard deviation

group I and IV being of a very statistical significance. The satura- x, expressed as a percentage, showed first few donations a slight but not nt increase, but in the last group x dropped to a very low figure (27.3), significantly lower than those of ther groups.

emoglobin values showed no signi- anges and were in all groups well ormal limits, as donors with signs a in the form of a decreased hemo- oncentration were not included in erial. The protein concentration of as normal and almost the same in all ups.

hus obvious from the above that a al of 350 ml of blood 1—4 times year did not provoke any signs of remia in this material. However, e or more units of blood were re- er year the serum iron showed a tendency to drop, accompanied by aneous rise of the TIBC, resulting ration index well below that of the naterial.

quently, if no supplementary iron is e intervals between the bleedings of male blood donors should be kept at ee months.

SUMMARY

iron, total iron-binding capacity and saturation index have been n 136 male blood donors, all having lobin concentration within normal > 12.3 g per 100 ml). None of the ad received supplementary iron.

removal of blood at a rate of 1 to 350 ml) per year the serum iron showed a significant increase. At a donation rate of 5 to 6 units or more per year, the serum iron dropped to an average value below that of the normal material, though still within the normal range. In these donors the TIBC showed an increase, resulting in a lowered saturation index with an average value significantly beyond the normal range. This is interpreted as indicative of a negative iron balance and a latent sideropenia in the group that was bled 5—6 times yearly. A safe schedule for healthy male blood donors would thus comprise not more than four donations yearly of 350 ml each, unless prophylactic iron is given regularly.

REFERENCES

Berde, B., Holländer, L., Undritz, E. & Zehnder, K.: Serumeisenstudien bei Blutspendern. Schweiz. Med. Wchnschr. *85*, 936, 1955.

Davis, F.: The effects on blood donors of regular removal of blood. Med. J. Austral. *42*, 384, 1953.

Hagberg, B.: The iron-binding capacity of serum in infants and children. Acta Paed., suppl. *93*, 1953.

Haskins, D., Stevens, A. R., Finch, S. & Finch, C. A.: Iron metabolism. Iron stores in man as measured by phlebotomy. J. Clin. Investigation *31*, 543, 1952.

Kaldor, I.: Studies on intermediary iron metabolism. II. Variations of the serum iron value in blood donors and control subjects. Austral. J. Exper. Biol. *31*, 49, 1953.

Laurell, C. B.: Studies on the transportation and metabolism of iron in the body. Acta Physiol. Scandinav. *14*, suppl. 46, 1947.

Moore, C. V.: The importance of nutritional factors in the pathogenesis of iron-deficiency anemia. Am. J. Clin. Nutrition *3*, 3, 1955.

Möller, H.: Untersuchungen am Blutspender insbesondere Serumeisen- und Serumeiweiss-Bestimmungen. Ztschr. f. d. ges. Med. *7*, 341, 1952.

Norberg, B. & Ramgren, O.: Haemoglobin, serum iron and plasma protein values in blood donors. Nord. Med. *56*, 1649, 1956.

Rath, C. E. & Finch, C. A.: Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXXVIII. Serum iron transport, measurement of iron-binding capacity of serum in man. J. Clin. Investigation *28*, 79, 1949.

Remy, D., Goldeck, H. & Krüger, E.: Eisenmangel bei häufigem Blutspenden. Deut. Med. Wchnschr. *77*, 1253, 1952.

Sköld, E. & Waldenström, J.: Hämoglobin-serumjärnvärden hos blodgivare. Ett bidrag den s. k. blödningsanämiens patogenes. Sv. Läk.tidn. *37*, 1359, 1940.

Vahlquist, B.: Das Serumeisen. Eine pädiatr. klinische und experimentelle Studie. Paed., suppl. 5, 1941.

. J. Clin. & Lab. Investigation 67—71, 10, 1958.

TERMINATION OF MAXIMUM WORKING CAPACITY T DIFFERENT AGES IN WORK WITH THE LEGS OR WITH THE ARMS

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(Received for publication October 26, 1957)

apacity for doing hard muscular work expressed as the "aerobic capacity" d & Ryhming 1954) *i. e.* the maxi-2-uptake obtainable during work in or semi-steady state. The determina-he aerobic capacity by pushing the or the patients to the limits of their power is in many respects undesir-d Åstrand & Ryhming, therefore, d a nomogram by means of which it le with fair accuracy to predict the capacity from measurements made submaximal work. The nomogram — o far only can be used on young years) males and females — is based fact that the heart rate during work cases is rectilinearly correlated to k intensity or to the O_2-uptake, cf. trand (1952, 1956) and Christensen

lata that form the basis for the nomo-ere collected by Åstrand (1956) and from experiments on young subjects ard work with the large muscles of er extremities, *e. g.* running on the ll, bicycling or doing the step test. practical work it is often desirable estimate the aerobic capacity of per-older ages, and as sometimes in work rtly paralyzed patients it is necessary to use arm-work as a test, it was attempted to modify the method of Åstrand & Ryhn-ning in such a way that this could be ac-complished.

The first of these modifications is rather simple. If heart rate and O_2-uptake during work are rectilinearly correlated, it follows that the increase in O_2-uptake above the resting value (extra O_2) must be propor-tional to the corresponding increase in pulse rate above the resting value,
or:

$$\text{Extra } O_2 = C \times \text{pulse increase.}$$

For any subject C can be determined from measurements of extra O_2 and pulse increase at any convenient, submaximal work, and the extra O_2-uptake during maximum work ($\Delta O_{2\,max}$), *i. e.* at maximum pulse rate, can be determined as

$$\Delta O_{2\,max} = C \times \Delta P_{max}$$

where ΔP_{max} denotes the maximum in-crease in pulse rate above the resting value. ΔP_{max} is the difference between the resting pulse rate and the maximum pulse rate dur-ing work. For men between 20 and 60 years of age the resting pulse rate can without great error be set to 60, and for women be-tween 20 and 60 years to 70. The maximum working heart rate decreases with age, *viz.* from about 195 at 20 years to about 160 at

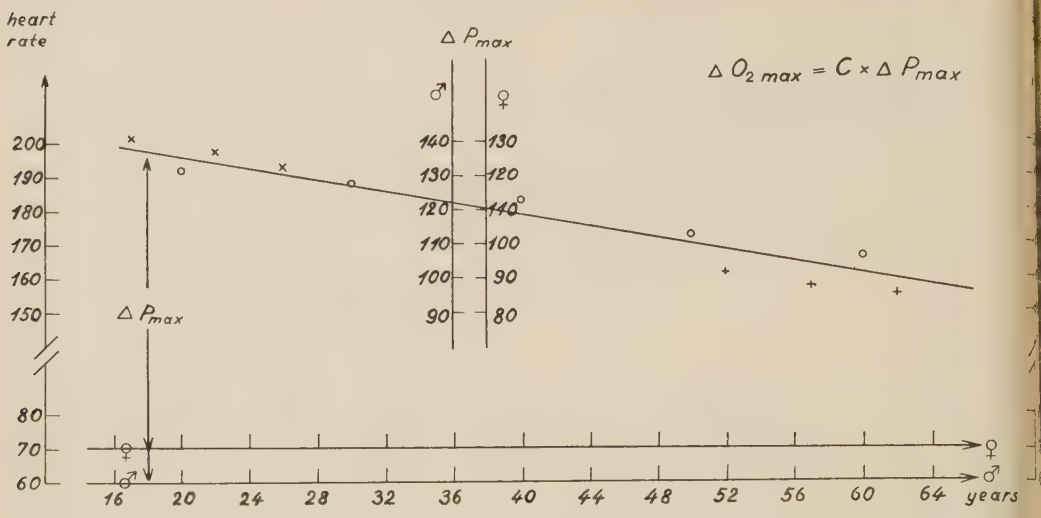


Fig. 1. Curve and formula for determining ΔP_{max} and $\Delta O_{2\,max}$ at varying ages.
C is $\frac{\text{increase in } O_2\text{-uptake}}{\text{increase in heart rate}}$ at any submaximal rate of work.
O data from curve of S. Robinson (1938) based on 79 male subjects.
× data from Åstrand (1956) on 105 male and female subjects.
+ data from Åstrand & Åstrand (personal communication) on 73 male subjects.

the age of 60. A curve based on observations by Robinson (1938), Åstrand (1956) and Åstrand & Åstrand (personal communication) is presented in Fig. 1 from which ΔP_{max} can be read for any age between 20 and 60 years. The aerobic capacity (Åstrand) is then

$$\text{resting } O_2\text{-uptake} + \Delta O_{2\,max}$$

where the resting O_2-uptake can either be determined directly, read from tables of standard metabolism or, roughly, be set at 250 ml/min. for men and 200 ml/min. for women.

In cases where the patients are unable to walk but have full control of shoulders and arms, the work can be performed on a hand-driven tricycle — *e. g.* on a motor-driven treadmill with adjustable incline.

In order to investigate whether formu of the above kind could justifiably be us also in such cases, a series of experime were performed to find out:

1) if the same maximum heart rate co be obtained with arm-work as with leg-wo

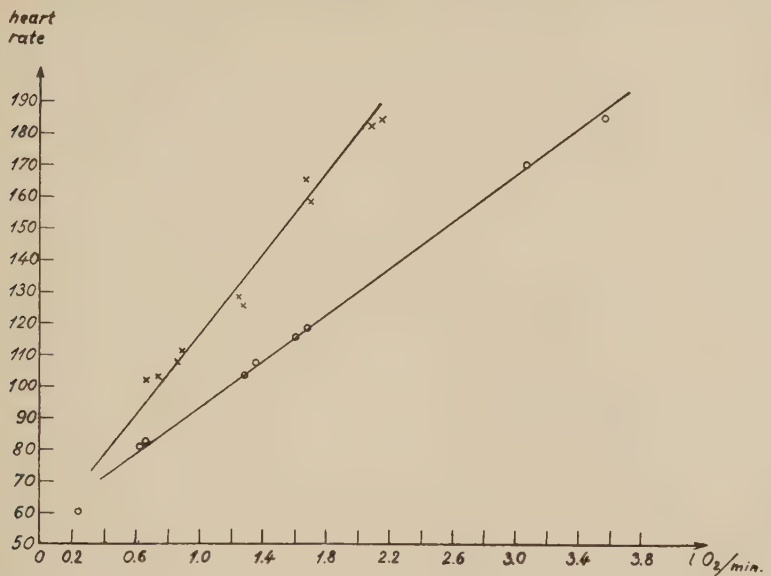
2) if the heart rate-O_2-uptake relat was rectilinear with arm-work and

3) to see if the slope and origin of heart rate-O_2-uptake curve were differen arm-work from those found in leg-work.

METHODS

The experiments were performed under st dard conditions (fasting, preliminary rest for to 45 minutes) on 11 normal men aged 20 29 years.

The subjects walked on the treadmill for



Heart rate in relation to O_2-uptake during work performed with the legs (o) and with the arms (×). Subject I. H. 29 years.

drove the tricycle for arm-work at dif-lines of the treadmill so that complete O_2-uptake curves were obtained for both ork. The oxygen uptake was determined uglas-bag method and the pulse rate the electrocardiogram (chest lead) after r semi-steady state had been reached, fter 5 to 10 minutes of work. At the st rates of work a true steady state was d for the heart rate, which continued to so after the first 10 minutes of work. rates used were those registered during riods of collecting expired air, *i. e.* be-10th and the 15th minute of work.

SULTS AND DISCUSSION

shows a representative couple of e-O_2-uptake curves while Fig. 3 ail the measured data from eleven

ared from the results that 7 of the ts attained heart rates higher than per minute during work with the arms, while 10 did so during work with the legs. Two of the four subjects who did not reach this pulse level during arm-work were definitely unable to work harder than they did, while the other two apparently were not pushed to the limit of their capacity.

All the curves showed a practically rectilinear relationship between heart rate and O_2-uptake during arm-work as during leg-work, with a tendency in 2 cases of each kind of work to level off when the heart rate exceeded 180. The slope of the curves for arm-work was, as seen in Figs. 2 and 3, always steeper than in those for leg-work.

The extension of the curves back to resting O_2-uptake and pulse rate (lying horizontally) was in 8 of the 11 subjects doing arm-work a straight line. The remaining 3 subjects showed a more or less upward displacement of the point of origin for the curves for arm-work.

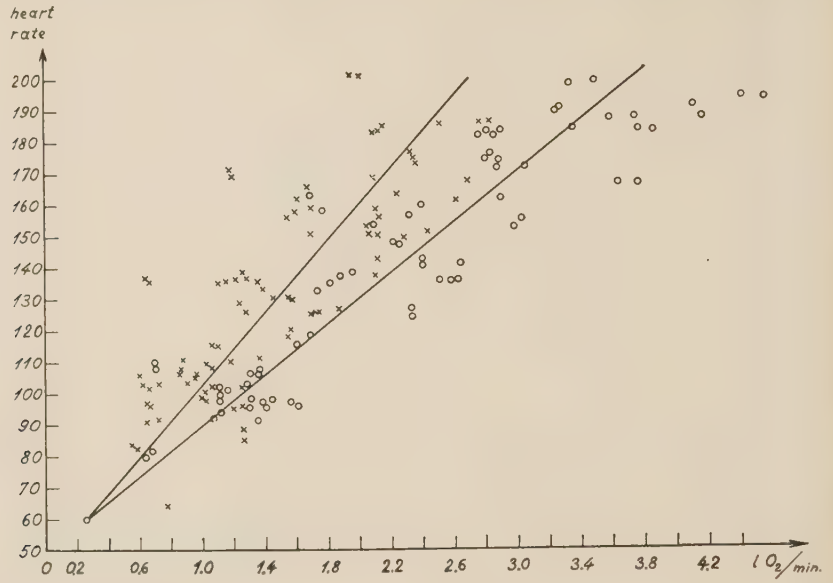


Fig. 3. Composite curves of heart rates in relation to O_2-uptake for arm-work (×) and leg-work (O) in 11 subjects.

It must be assumed that this is the effect of a hydrostatically caused shift of blood to the more or less passive legs in the sitting position on the tricycle. It has previously been found (*e. g.* Nielsen & Fridrichsen, 1938; Asmussen & Christensen, 1939) that this effect is added to the effect of work on the pulse rate.

From the experiments it seems justifiable to conclude that a fairly accurate estimate of the maximum capacity for arm-work can be deduced from submaximal work performances in the same way as for leg-work, *e. g.* by the formula on page 67 and Fig. 1.

It is, however, not possible to estimate the total aerobic capacity for leg-work from experiments with arm-work and *vice versa.* Further it must be expected that work with still smaller muscle groups cannot be used in the way described here, because the m mum pulse rate 170—200 cannot be att while working with too small a muscle g The mass of muscles used in propell tricycle is probably the limit of what i cessary to force the heart rate up to m mum values.

The reason why the pulse rate at a g O_2-uptake is higher in arm-work tha leg-work cannot be given. The hydro factor, mentioned above, was in a few subjects responsible for a slight increa the resting value, but cannot explai steeper slope of the pulse-O_2-uptake c in arm-work.

The smaller "oxygen-pulse" (O_2-u per heart beat) would suggest a sm stroke volume of the heart during arm presuming the cardiac output is the sam

as for leg-work at the same oxy-
. Whether this latter assumption
not finally settled, but a somewhat
roke volume during work with
le groups may be understandable
dering the smaller capacity of the
mp in arm-work as compared to

SUMMARY

data available from the literature,
and a curve are presented from
maximum extra oxygen uptake
k can be determined from meas-
made during submaximal work
).
robic capacity" (Åstrand) thus
mated for men and women from
ears of age.
rther been shown by experiments
mal male subjects that the same
n be used also when the subjects
vork with their arms on a tricycle.
ns the possibility of determining
"aerobic capacity" on patients *e. g.* with paralysis of the lower limbs.

In normals, the "aerobic capacity" is considerably lower in arm-work than in leg-work.

REFERENCES

Asmussen, E. & Christensen, E. H.: Einfluss der Blutverteilung auf den Kreislauf bei körperlicher Arbeit. Skandinav. Arch. Physiol. *82,* 185, 1939.

Åstrand, P.-O. & Ryhming, I.: A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. J. Appl. Physiol. *7,* 218, 1954.

Åstrand, P.-O.: Experimental studies of working capacity in relation to sex and age. Munksgaard. Copenhagen, 1952.

— Human physical fitness with special reference to sex and age. Physiol. Rev. *36,* 307, 1956.

Christensen, E. H.: Die Pulsfrequenz während und unmittelbar nach körperlicher Arbeit. Arbeitsphysiologie 4, 453, 1931.

Nielsen, M. & Fridrichsen, E.: Eine Modifikation des Krogh'schen Fahrradergometers. Skandinav. Arch. Physiol. 78, 76, 1938.

Robinson, S.: Experimental studies of Physical fitness in relation to Age. Arbeitsphysiologie *10,* 251, 1938.

Scandinav. J. Clin. & Lab. Investigation 72—80, 10, 1958.

A METHOD FOR QUANTITATIVE EVALUATION OF RED CELL TURNOVER IN HUMANS[1]

I. NORMAL VALUES

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(Received for publication November 13, 1957)

Simultaneous analysis of rates of production and destruction of erythrocytes in humans has been made by utilizing the differential agglutination technic (DeGowin, Ellis, Sheets, Hamilton & Janney, 1954). Erythropoiesis, red cell production and destruction have been evaluated quantitatively by employing hematological data, bone marrow examination, fecal urobilinogen excretion and radioactive iron turnover with or without direct determination of red cell survival (London, Shemin, West & Rittenberg, 1949; Crosby & Akeroyd 1952; Loeb, Moore & Dubach, 1953; Seip, 1953; Giblett, Coleman, Pirzio-Biroli, Donohue, Motulsky & Finch, 1956; Joske, McAlister and Prankerd, 1956; Bush, Aschenbrucker, Cartwright & Wintrobe, 1956).

For the purpose of evaluating the relative importance of inadequate production and increased destruction of red cells in clinical states where the total red cell mass is continuously changing, a new approach was found to be necessary. A method, base Cr^{51}-tagging of the individual's own cells combined with repeated determina of total circulating red cell volume wa veloped and evaluated in clinical and ex mental studies of burn anemia.

The present paper describes the te and the normal range of values obtain healthy humans.

METHODS

Hematocrit as per cent (Hct) and hemo as grams per 100 ml blood (Hgb) were deter by standard methods (Birkeland, 1958, b).

Total red cell volume (RCV) was calc from Hct and plasma volume (PV), or i determined by using a modification of th method of Reeve & Veall (1949), (Birk 1958, d). Where a double red cell tag (P Cr^{51}) was employed, the sample (5 ml of hem blood) activity (C_{sc}) was first determined in scintillation counter (Nuclear). Thereafter 5 buffer (Reeve & Veall, 1949) was added to th sample and its activity (C_{gm}) in a liquid Müller tube (Veall, 1948) was obtained.

Activity of the individual isotopes wa culated as follows (expressions rounded):

$$Cr^{51}\text{-act. (c/min..)} = C_{sc} - aC_{gm},$$

$$P^{32}\text{-act. (c/min)} = \frac{C_{gm} - b\,C_{sc}}{1 - b \times a} = C_{gm}$$

The factors a and b, determined experimentall found to be 0.08 and 0.002 respectively.

[1] This study was conducted under U. S. Public Health Grant H-1142 on a Research Fellowship in Surgery at the Medical College of Virginia, 1952—1955.

The preparation of the data was supported by a grant from the Norwegian Research Council for Science and the Humanities.

more samples were counted for each RCV was determined.

Cr^{51}-survival was determined and Cr^{51}-survival" was calculated as descri- nd, 1958, c). Sampling and transfusions ed and Balance Index (B. I.) was cal- eported (Birkeland, 1958, b). The B. I. roduction of red cells per day as per- he existing red cell volume at selected ssuming a normal rate of destruction fe span of 115 ± 15 days) and with nade for sampling and transfusions.

ON OF RED CELL PRODUCTION AND DESTRUCTION

ained by using the methods refer- e included red cell survival curve, RCV at intervals and quantities of emoved as samples and given as s. As described elsewhere (Birke- , c), the observed Cr^{51}-survival ealthy individuals with correction sampling were adequately fitted rst 50 days by the equations

$$N_o \left(1 - \frac{t}{115}\right) e^{-0.0136\ (t+1)}$$

$$= N_1 \left(1 - \frac{t-1}{114}\right) e^{-0.0133\ (t-1)}$$

stands for total Cr^{51}-activity con- CV one hour after infusion of tag- N_1 and N_t are the corresponding ay one and on day t after infusion. urves of the Ashby type ("Cor- -survival") were obtained by cor- the elution process, *i. e.* dividing ed N_t-values by the exponential quations I and II.

rage (or mean) red cell survival e determined from survival curves o the methods developed by Dorn- 1), as applied to the individual's own red cells instead of transfused cells (Hughes Jones & Mollison, 1956). During a steady state (*i. e.* RCV constant) production of red cells may be estimated when the average survival time is known (London *et al.*, 1949; Crosby *et al.*, 1952; Mollison, 1956).

In major burns and other trauma patients studied a steady state was rarely observed. In burns red cell destruction curves often showed steeper slopes during certain periods. From a complete curve of this type the average life span was not readily determined. Red cell balance data indicated that the rate of destruction could be different during various clinical phases. For these reasons it was found desireable to analyze the different phases separately with respect to destruction and production. The practical problem was thus reduced to analyzing separately the shorter periods during which the survival curve showed a substantially rectilinear course.

RCV at any time will be directly dependent upon the two main factors: red cell production (release from bone marrow) plus given transfusions on the one side — and red cell destruction within the organism plus external blood loss on the other.

Red cell turnover is defined as the total quantity of circulating red cells removed from and added to the circulatory system in unit time. The RCV is throughout expressed as volume of packed cells. The packing is defined by the hematocrit. The unit time is given in days. Based on the following two assumptions an attempt was made to estimate the red cell turnover in quantitative terms:

1) The elution rate of Cr^{51} from autotransfused, viable red cells is constant from

the first post-transfusion day up to about 50 days and equal to the rate observed in healthy controls.

2) Destruction rate of autotransfused Cr^{51}-tagged red cells is at all times after the first post-transfusion day representative of the corresponding rate for the individual's total circulating red cell volume. There must be no difference between tagged and untagged cells with respect to intracellular defects or age distribution.

The following derivation is described in general by using k for any of the constants in the exponential factors in Equations I and II. RCV determined at selected time zero is RCV_0 and on day t thereafter it is RCV_t. Cr^{51}-activity per ml of red cells is C_0 at time zero and C_t on day t. t indicates the number of days after any selected zero time (at which cell survival is taken to be 100 per cent), and t is less than 50 days. — A summary diagram is shown in Fig. 1.

Estimation of red cell destruction.

For this purpose the term destruction includes destruction within the organism as well as uncontrolled external blood loss. The average survival time (Tm) for red cells during the selected period was taken as the main characteristic for describing rate of destruction. A rectilinear survival curve during such a period may be extrapolated to reach the time axis, and the number of days elapsing from initial (100 per cent) to zero value may be read off from this axis as Tm. It is pointed out that the selected N_0 should be charted as 100 per cent and N_t/e^{-kt} as per cent of N_0 on a linear scale in order to determine Tm graphically by extrapolation.

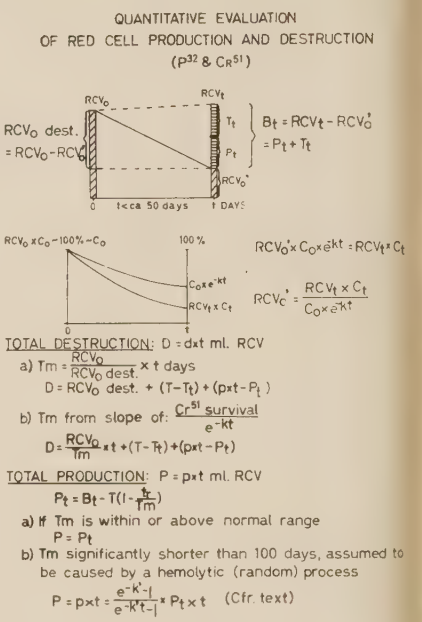
QUANTITATIVE EVALUATION
OF RED CELL PRODUCTION AND DESTRUCTION
(P32 & Cr51)
RCV0
RCVt
RCV0 dest.
= RCV0 - RCV0'
Tt
Pt
Bt = RCVt - RCV0'
= Pt + Tt
RCV0'
0
t<ca 50 days
t DAYS
RCV0 x C0 = 100% - C0
100 %
C0 x e-kt
RCVt x Ct
RCV0 x C0 x e-kt = RCVt x Ct
RCVc' = RCVt x Ct / C0 x e-kt
TOTAL DESTRUCTION: D = d x t ml. RCV
a) Tm = RCV0 / RCV0 dest. x t days
D = RCV0 dest. + (T - Tt) + (p x t - Pt)
b) Tm from slope of: Cr51 survival / e-kt
D = RCV0 / Tm x t + (T - Tt) + (p x t - Pt)
TOTAL PRODUCTION: P = p x t ml. RCV
Pt = Bt - T(1 - t/Tm)
a) If Tm is within or above normal range
P = Pt
b) Tm significantly shorter than 100 days, assumed to be caused by a hemolytic (random) process
P = p x t = e-k't - 1 / e-k't - 1 x Pt x t (Cfr. text)

Fig. 1.

Within practical working limits, multi values were obtained on the curve in orde ascertain its true course.

A RCV-determination could not be m at all sampling points where C_t was me ured. In these instances RCV_t was estima by linear interpolation between the prec ing and the subsequently determined RC values. — For practical purposes it found practicable in most instances to data for analysis from two points only, (at the beginning and at the end of period). At these points RCV_t as wel C_t had to be determined.

Calculations were carried out as follo

If Cr^{51}-activity was lost by the proce elution only (*i. e.* no loss by aging or rand destruction), then $N_t = RCV_t \times C_t = RCV_0 \times C_0 \times e^{-kt} = N_0 \times e^{-kt}$

in two points only. If at any time, RCV_o was left of the original RCV_o (RCV_o'dest.) of RCV_o must have (by any mechanism) — equal to

$$V_o \text{ dest.} = RCV_o - \frac{RCV_t \times C_t}{C_o \times e^{-kt}}$$

$$= RCV_o - \frac{N_t}{C_o \times e^{-kt}}$$

of production and destruction of cells during O—t days.

his expression (Equation III) it is if C_t is greater than $C_o \times e^{-kt}$, raction of untagged cells was de- an of tagged ones.

iple of significant quantity (S ml) ls of known activity (C_s) per ml ved at any time ($t—t_s$), correction mpling was included in Equation then became

$$V_o \text{dest} = \frac{V_t + S \times C_s\left[1-(1-\frac{N_t}{N_0})\frac{t_s}{t}\right]}{C_o \times e^{-kt}}$$

red cell survival time (Tm) was

$$= \frac{RCV_o}{RCV_o\text{dest}} \times t$$

cates the volume of cells remaining from a volume (T) of untagged s (Birkeland, 1958, b) given as or- od transfusion at any time ($t—t_T$). nts the total volume present at time gged cells that were added to the during O—t days.

estruction (D) of cells during O—t be

$= d \times t$

dest + $(T — T_t) + (P — P_t)$

under a) in Fig. 1 where

$1 — \frac{t_T}{Tm}$), and $P = p \times t$

$p =$ ml of red cells released to the circulation per day on the average (*i. e.* true production).

b) *From the slope of corrected Cr^{51}-survival.* If multiple points did not form a well characterized curve of the types mentioned below, a straight line drawn to the best fit of all points involved was assumed to describe the most correct slope. If any of the two end-points (N_o or N_t/e^{-kt}) deviated clearly from this line, the line itself was extrapolated for determination of Tm as previously described.

The resulting

$$\text{(VII)} \quad D = \frac{RCV_o}{Tm} \times t + (T—T_t) + (P—P_t)$$

as under b) in Fig. 1.

Especially when a larger portion of the curve was included, it happened that a more well-defined initial slope was present of the type indicating an intrinsic red cell defect, or that the curve formed close to a straight line when plotted on semi-logarithmic paper, indicating random destruction (Dornhorst, 1951). In these instances the Tm was also estimated according to the methods of Dornhorst (1951). The expression for D would be as Equation VII with correct substitution for Tm.

In this presentation values for D were calculated from Equation VI.

Estimation of red cell production (release from bone marrow).

From the upper right part of Fig. 1 it is seen that

$$\text{(VIII)} \quad P_t = B_t—T_t = B_t—T \ (1—\frac{t_T}{Tm})$$

If no transfusion was given ($T = O$), the

volume B_t must be the result of untagged cells that were produced and not destroyed during O—t days, *i. e.* B_t equal to P_t.

a) *When Tm was within normal range* (100—130 days), none of the newly formed cells were destroyed by day t (as t<50 days), and total production during O—t days would be

$$(IX) \qquad P = p \times t = P_t$$

If Tm was shortened because of external blood loss, P was taken to be equal to P_t — realizing that this might result in a slight underestimation of P.

b) *When Tm was significantly shortened* and external blood loss was excluded, the estimation of P from P_t was no longer straight forward.

If a random removal of red cells irrespective of their age is assumed to be taking place, the value of P_t will be more or less an underestimation of P depending on whether the random destruction process is more or less active respectively. The true production P should include all cells that were released to the circulation during t days, regardless of whether they were present in circulation at time t or not.

Where the curve for corrected Cr^{51}-survival clearly indicates random destruction, its individual values may be divided by $(1 - \frac{t}{115})$ to eliminate the effect of normal aging. If the resulting values (representing the random process) fall on a straight line on semilogarithmic paper, the half time may be determined graphically and the corresponding exponential constant (k′) can be calculated. From such a set of data, P may be calculated as follows:

$$P_t = p\ (e^{-k'(t-1)} + e^{-k'(t-2)} + \cdots$$

which gives:

$$(X) \quad P = p \times t = \frac{e^{-k'} - 1}{e^{-k't} - 1} \times P_t \times$$

as shown in Fig. 1 (Production, b).

In general, when data are available for vival curve, RCV_o and RCV_t as well a cells removed and transfused, true produc (P) may theoretically be estimated u certain conditions (example: Equation when the process which is eliminating new cells (released during time O—t) operating at a uniform rate, and this rate be adequately expressed numerically.

Quantitatively, an intracellular defect co not be shown to play more than a minor r in burns (Birkeland, 1958, d). The po bility that the shortened Tm in these patie was largely due to a random destruc mechanism was not unlikely. If such an sumption is accepted, P may be estimated cording to Equation X, although only points are used. However, in burn patie where destruction was considerably inc sed, values for P_t were usually very low that the more elaborate calculation of P (cording to Equation X) was of limited p tical importance. Data on production, culated according to Equation IX in patie must be considered with this limitation mind. — The data in Fig. 2 were obtaine using Equation IX.

REFERENCE VALUES

Normal destruction may be expressed

a) Tm = 115 days (Birkeland, 1958, b.

or

b) $\frac{\text{Existing RCV at time zero (}i.\,e.\text{ RC}}{115}$

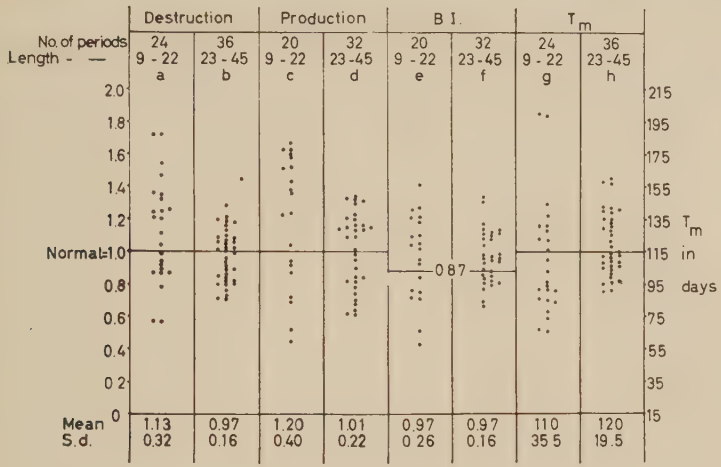

Fig. 2.

er value (b) is not an adequate if transfusions are given during undergoing analysis — as destruc- nsfused cells will not be included. eason Tm was preferred for refer- in patients as it depends on the truction only.

production was expressed as

$$\frac{\text{Normal RCV}}{115} \quad \text{ml/day}$$

trol values for RCV obtained in lividuals (Birkeland, 1958, d) were ormal:

les 28.5 ml per kg body weight.
nales 25.0 ml per kg body weight.

riation was considerable between healthy individuals with respect to ressed as ml per kg body weight. ıtly, the reference value for pro- subject to a similar wide normal

RESULTS

In order to establish a normal range for the method, data were obtained in healthy controls (volunteers) according to methods described under a) for destruction and production. The raw data from 11 individuals are given in Table I.

For B. W. data on production before day 23 were excluded because a phlebotomy (290 ml red cells) was made one day prior to zero time.

These subjects are the 10 controls in whom Cr^{51}-disappearance from red cells (Equations I and II) was determined, and in addition one healthy male (G. B.) who received a transfusion from a patient. This patient suffered from a minor superficial burn (15 per cent scald), and had her red cells tagged and infused into the volunteer. The Cr^{51}-survival fell within normal range, and the data were therefore included in the control group.

By using Equations I and II and correcting (Equation IV) for sampling, the calculated rate of destruction is in Fig. 2 expressed as

Table I. *Data from 11 healthy controls.*

Subject Body weight	Days after transfusion	Hct	Cr^{51} activity C/min. per ml cells	RCV T-1824-technic	Observed Cr^{51}-survival per cent	Sampling red cells
B. W. 72 Kg.	0	43.8	732.4	2052	100.0	20
	2	42.2	688.6	2116	97.9	29
	9	41.3	562.9	2181	82.5	25
	23	42.1	375.2	2363	59.7	46
	44	45.5	204.2	2452	33.7	
A. K. 75 Kg.	0	37.3	224.2	1755	100.0	27
	6	36.7	189.5	1747	83.8	24
	23	36.7	135.6	1750	60.3	13
	44	40.8	72.0	1669	30.6	
B. C. 70 Kg.	0	39.5	260.4	1913	100.0	29
	5	39.2	224.6	1983	87.0	24
	26	38.6	130.1	1987	51.9	31
	44	42.9	75.0	1931	29.1	
A. G. 76 Kg.	0	37.5	603.2	2065	100.0	27
	5	37.3	532.2	2030	86.7	23
	26	38.0	332.0	1996	52.2	23
	44	38.2	224.8	1846	33.3	38
H. H. 61 Kg.	0	37.7	2006.0	1917	100.0	40
	35	38.6	830.0	1815	39.2	
G. D. 62 Kg.	0	35.5	460.8	1463	100.0	37
	41	35.4	171.7	1498	38.2	
C. M. 46 Kg.	0	35.2	434.4	1250	100.0	40
	38	36.7	166.9	1334	41.0	
E. C. 77 Kg.	0	43.6	1692.0	1929	100.0	44
	15	43.8	1043.0	2098	67.0	18
	33	40.0	637.0	2148	41.9	
W. S. 56.4 Kg.	0	33.7	2153.0	1498	100.0	25
	6	38.6	1883.0	1481	86.5	12
	11	39.4	1645.0	1501	76.6	33
	24	38.3	1097.0	1536	52.2	16
	38	36.3	750.0	1559	36.2	12
	45	35.9	617.0	1686	32.3	
O. S. 59 Kg.	0	43.5	4653.0	1745	100.0	19
	2	43.3	4290.0	1771	93.2	15
	7	39.8	3672.0	1821	82.3	17
	16	41.9	2872.0	1789	61.6	33
	38	41.1	1827.0	1825	41.1	13
	45	44.2	1350.0	1758	29.2	
G. B. 80 Kg.	0	44.9	246.7	2238	100.0	10
	2	45.8	226.2	2351	96.1	35
	15	52.3	175.7	2186	69.6	61
	31	43.4	116.8	2232	47.2	29
	38	46.2	102.0	2214	40.9	

mns g and h) and as a fraction of columns a and b). Calculated rate tion was expressed as a fraction of ference value (columns c and d). B. I. are found in columns e and f. ttom of Fig. 2 mean values and leviation (s. d.) are listed for each

were calculated for all periods be- two measurements up to 45 days sion in order to give equal weight iods. The spreading of the results to be dependent on the length of al between the two measurements periods of 2—8 days duration the from normal were considered to eat for the present purpose. The n of the method was therefore re- periods lasting 9—45 days. For plication, periods of 10—20 days were often of great interest and in- e grouping of data as shown in

DISCUSSION

ted that higher values for produc- destruction were observed during r periods. This could suggest that n or cell loss were greater during ods. However, on inspection of the points, the higher values were be spread over the entire course of s. Therefore the limited number ations seem to be the likely ex-

newhat higher mean value of 0.97 (theoretical normal 0.87, range 0) may be due to stimulated pro- caused by the repeated sampling

The range of obtained normal values is fairly wide for the shorter periods. For the longer ones (23—45 days) the range is reduced to about half, and is not far from the expected variation. When Tm was obtained from the slope of multiple points, the normal range gradually decreased with the number of points included. Eventually, by using all points available, the range of Tm fell within 99—127 days (Birkeland, 1958, c). If normal Tm is assumed to be 115 ± 15 days, it is emphasized that during a steady state the expected normal range for production and destruction should be 0.87—1.13.

When the shorter periods were evaluated as parts of the total curve, the general trend of red cell turnover in relation to clinical events was reasonably well described as shown in the following paper (Birkeland, 1958, a).

The foundation of the underlying assumptions in healthy subjects is as follows: The first assumption was derived directly from the observed data (Birkeland, 1958, c). The second assumption was based on the common observation that all cells in circulating blood show the same rate of destruction within accepted range (Crosby, 1954). — The role of these factors in pathological states is discussed in the following paper.

SUMMARY

A method is described for the quantitative evaluation of red cell turnover in man. The method is based on the assumption of a constant elution rate of Chromium from intact red cells, and on repeated determinations of circulating red cell volume. Normal values are reported as obtained in eleven healthy subjects.

REFERENCES

Birkeland, S.: a) A method for quantitative evaluation of red cell turnover in humans. II. Clinical application. Scandinav. J. Clin. & Lab. Investigation *10,* 81, 1958.

— b) A red cell balance index — B. I. Scandinav. J. Clin. & Lab. Investigation 1958. In press.

— c) The use of the isotope Cr^{51} for studying destruction of red cells in healthy humans. Scandinav. J. Clin. & Lab. Investigation 1958. In press.

— d) A study in burns. In preparation.

Bush, J. A., Ashenbrucker, H., Cartwright, G. E. & Wintrobe, M. M.: The anemia of infection. XX. The kinetics of iron metabolism in the anemia associated with chronic infection. J. Clin. Investigation *35,* 89, 1956.

Crosby, W. H.: The hemolytic states. Bull. N. Y. Acad. Med. *30,* 27, 1954.

— & Akeroyd, J. H.: The limit of hemoglobin synthesis in hereditary hemolytic anemia. Am. J. Med. *13,* 273, 1952.

DeGowin, E. L., Ellis, J. A., Sheets, R. F., Hamilton, H. E. and Janney, C. D.: Studies with inagglutinable erythrocyte counts. IV. Graphic analyses of rates of production and destruction of human erythrocytes. J. Clin. Investigation *33,* 163, 1954.

Dornhorst, A. C.: The interpretation of red cell survival curves. Blood *6,* 1284, 1951.

Giblett, E. R., Coleman, D. H., Pirzio-Biroli, G., Donohue, D. M., Motulsky, A. G. & Finch, C. A.: Erythrokinetics: Quantitative measurements of red cell production and destruct normal subjects and patients with a Blood *11,* 291, 1956.

Hughes Jones, N. C. & Mollison, P. L.: The i pretation of measurements with Cr^{51}-lab red cells. Clin. Sci. *15,* 207, 1956.

Joske, R. A., McAlister, J. M. & Pran T. A. J.: Isotope investigations of red production and destruction in chronic r disease. Clin. Sci. *15,* 511, 1956.

Loeb, V. Jr., Moore, C. V. & Dubach, R.: physiologic evaluation and managemen chronic bone marrow failure. Am. J. Me 499, 1953.

London, I. M., Shemin, D., West, R. & Ri berg, D.: Heme synthesis and red blood dynamics in normal humans and in sub with polycythemia vera, sickle-cell anemia pernicious anemia. J. Biol. Chem. *179,* 1949.

Mollison, P. L.: Blood transfusions in cli medicine. Blackwell Scientific Publicat Oxford, England. Second ed. 1956.

Reeve, E. B. and Veall, N.: A simplified me for the determination of circulating red volume with radioactive phosphorus. J. Phy *108,* 12, 1949.

Seip, M.: Reticulocyte studies. Johan Gr Tanum Forlag, Oslo, Norway, 1953.

Veall, N.: A Geiger-Müller counter for measu the beta-ray activity of liquids. Brit. J. Ra *21,* 347, 1948.

J. Clin. & Lab. Investigation 81—90, 10, 1958.

METHOD FOR QUANTITATIVE EVALUATION OF RED CELL TURNOVER IN HUMANS[1]

II. CLINICAL APPLICATION

By S. BIRKELAND

urgical Research Laboratories, Medical College of Virginia, Richmond, Va., U. S. A. and ersity Institute for Experimental Medical Research, Ullevål Hospital, Oslo, Norway

(Received for publication November 13, 1958)

thod previously reported (Birke-, a) was designed for the study nemia as follows:

nically to define the need for blood t therapy at various clinical stages.

describe and interpret the frequent marked anemia tendency in terms s in red cell production and de- s related to clinical events.

sent communication describes the plication of the method with a dis- its usefulness. A complete report and experimental data will follow (Birkeland, 1958, d).

ATERIAL AND METHODS

n patients were treated according to described by Evans (1952).

hod used was in all details as reported going paper (Birkeland, 1958, a). Pro- destruction were calculated according s IX and VI respectively (Birkeland, nless otherwise stated. — Reticulocytes expressed as number per thousand of lls counted (3000) from a dry Brilliant slide (Ham, 1952). Total serum protein

udy was conducted under U. S. Public ant H-1142 on a Research Fellowship at the Medical College of Virginia,

paration of the data was supported by n the Norwegian Research Council for l the Humanities.

concentration was determined by the copper sulphate method (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald 1943—44).

RESULTS

In Tables I and II all raw data are presented with the exception of some values for column d obtained at times when no RCV-determinations were made. Days after injury or after infusion of tagged cells (T. E.) are listed in column a. Destruction is expressed as ml red cells per day (column g) and as Tm (column h), production as ml per day (column i) and as fraction of normal value (column j), red cell balance as B. I. (column k) and as ml (column l).

In the diagrams curve 1 describes observed Cr^{51}-survival (*i. e.* $N_o = 100$ per cent and N_t as per cent of N_o). Curve 2 was obtained from curve 1 by correcting for Cr^{51}-elution (Equations I and II, Birkeland, 1958 a). Curve 3 represents determined red cell volume (RCV_t) corrected for transfusions (*i. e.* minus T $(1-\frac{t_T}{Tm})$) and expressed as per cent of RCV_o. For simplicity no corrections for sampling were made for any of these curves (1, 2 or 3) — as sampling would affect all of them to the same extent. The RCV-curve gives the absolute values for determined RCV.

Now, if curve 2 is accepted as a true and representative description of the over-all rate of red cell destruction, then curves 2 and 3 must show the same slope during periods where no production of new cells is taking place. — Furthermore, during a period of steady state, curve 3 must be parallel to the 100 per cent horizontal dotted line. — Red cell balance is shown as positive and negative in relation to normal (*i. e.* B. I. = 0.87) and to zero value (dotted line). Production and destruction are charted as ml of red cells per day in positive and negative direction respectively with the resulting balance per day (*i. e.* production minus destruction) as doubly hatched areas. — O. R. stands for operation and + 500 for one pint of blood transfused. Figures in brackets are assumed values.

T. E. (Fig. 1, Table I), 54-year old white male.

The patient was admitted complaining of pain in the chest. Dyspnea and general edema were pre-

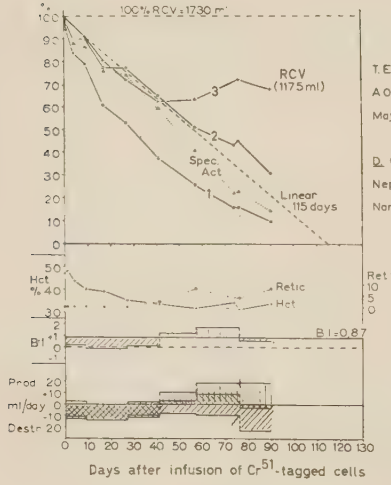

Fig. 1.

Table I. *Red cell turnover and balance in a patient with carcinoma of the lung and nephrotic syndrome.*

Days	Body weight	Hct	RCV P^{32}	PV T-1824	BV	Serum protein: Conc.	Serum protein: Total grams	B. Hct / Hct	C/min. per ml cells	Obs. Cr^{51}-survival per cent	Sample	Destruction: ml/day	Destruction: T_m	Production: ml/day	Production: Times normal	Balance: B. I.	Balance: ml RCV
a		b	c						d	e	f	g	h	i	j	k	l
0	64.5	47.3	1730	2220	3950	4.62	102.6	0.93	16325	100	45	13.2	127	2.4	0.2	0.15	—112
9	65.9	40.2	1578	2817	4395	5.38	151.6	0.89	14138	79.0	20						
17	-	39.3	1389	[2520]	[3909]	4.28	107.9	[0.91]	13574	60.9	23	14.0	157	0	0	—0.16	—293
27	64.6	35.3	1246	[2672]	[3918]	3.89	103.9	[0.91]	12068	53.2	26	11.6	105	2.0	0.1	0.07	—140
41	65.8	33.9	1085	2476	3561	5.00	123.8	0.90	9722	37.4	26	7.7	138	10.4	0.7	1.09	+ 38
57	50.5	31.9	1102	2805	3907	3.89	109.1	0.88	6691	26.1	30	9.6	111	18.6	1.3	1.66	+165
76	60.6	31.5	1243	3177	4420	4.28	136.0	0.89	3703	16.3	17	[9.6]	[80]	18.6	1.3	[0.87]	[44]

rdiac involvement was found. Carcinoma
was confirmed by needle biopsy. He
he picture of chronic nephritis with
yndrome showing a marked proteinuria
4), finely granulated and hyaline casts
ne. Serum protein values were low
with A/G ratio 0.3—0.4. Blood pressure
first study week fluctuated around
It went gradually down to 170/110 at
discharge. Pulse rate was 70—80 per
erature was normal or slightly sub-
oughout. Peripheral blood showed a de-
t and Hgb, a moderate (12,000) and
ukocytosis and a normal differential
t (P: 77—81, Eo: 1—2, L: 17—19,

s measured by the P^{32}-technic.
a indicate that red cell destruction
ntially normal as evidenced by the
during days 0—41 the slope of
and 3 are almost identical to the
ear aging process.
is patient Cr^{51}-survival during the
ays was expressed as activity per
ells (spec. act.), the observed curve
close to linear slope on semiloga-
aper with a half-time of about 55
h a slope would correspond to the
r^{51}-elution from normal red cells.
of this type could not be properly
d without simultaneous RCV-
ents.
lculated production was close to
ng the first 40 days. When RCV
60 per cent of the initial value (or
ately 17 ml/kg) production started,
locytes appeared in the peripheral

son for the stop in red cell produc-
not further investigated as the
s not available for re-examination
ne when the complete curve was

established. However, it may be noted that at the time when the production started, the edema subsided as shown by changes in body weight. During days 0—42 body weight fluctuated around 65.0 kg. On day 42 it was 63.2 kg, day 43 — 62.3 kg, day 44 — 60.2 kg, and later it stayed around 60 kg. A slight rise in PV after edema resorption was noted.

T. J. (Fig. 2, Table II). 21-year old colored female.

Four days prior to tagging of cells, the patient suffered a flame burn of legs and left arm of about 7 per cent (5 per cent of third degree) of the body surface.

The clinical course was essentially free from complications except for an unexplained spiking temperature during the second and third week after burning.

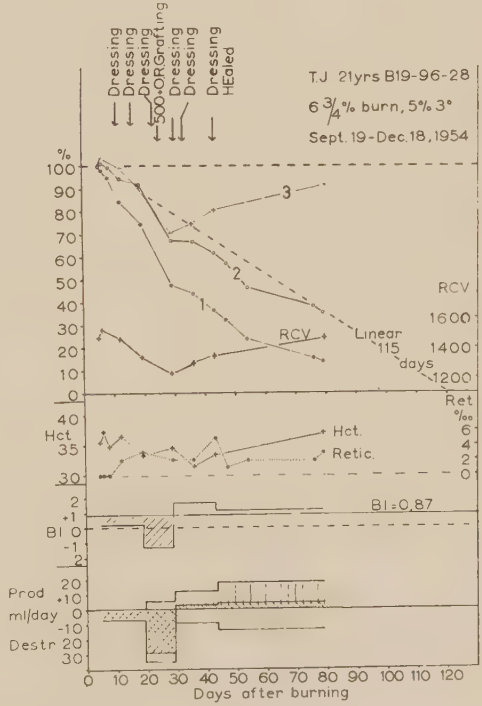

Fig. 2.

Table II. *Red cell turnover and balance in burns of minor (T. I.), moderately (H. E.) and very severe (M.W.) degree.*

Patient Days a	Hct b	RCV c	C/min. per ml cells d	Observed Cr^{51}-surv. per cent e	Sample f	Destruction ml/day g	Tm h	Production ml/day i	Production times normal j	B. I. k	Balance l
T. J.											
5	35.5	1469	655.8	100.0	18						
6	37.2	1523	620.0	98.0	15	7.5	200	0	0	0.19	−1
12	36.5	1458	566.5	85.7	15						
19	33.4	1341	545.2	75.9							
25	O. R. Grafting Transf. 213				26	35.3	40	5.6	0.4	—1.31	−1
29	34.4	1231	380.0	48.6	16						
36	31.5	1295	331.9	44.6	14	9.5	130	12.1	0.8	1.67	+1
43	33.6	1342	269.8	37.6	15	13.8	98	17.6	1.2	1.14	+1
79	37.0	1464	93.7	14.2							
H. E.											
1	54.5	2171	556.6	100.0	61						
2	Transf. 248					(21.9)					
2	55.7	2219	529.7	97.3	57	34.7	111	0	0	—0.31	−1
5	43.5	2096	507.7	88.1	60						
12	36.6	1951	434.5	70.2							
13	O. R. Debridement Transf. 235				48	106.9	21	17.9	0	—3.59	−3
19	O. R. Debridement Transf. 377										
20	46.3	1803	248.0	37.0	46						
27	O. R. Grafting Transf. [200]					25.7	89	0	0	—0.51	−1
33	34.0	1613	197.4	26.4	31						
39	32.3	1441	179.5	21.4							
45	33.3	1529			39						
51	O. R. Grafting Transf. 200				35					1.95	+1
56	35.4	1708			32						
62	34.6	1706									
	Wound healed				16					1.95	+1
76	36.3	1950									

Table II (cont.)

Hct b	RCV c	C/min. per ml cells d	Observed Cr^{51}-surv. per cent e	Sample f	Destruction ml/day g	Tm h	Production ml/day i	Production times normal j	B. I. k	Balance ml RCV l
nsf.	213									
4.0	1042	2019	100.0	72	83.0	15	11.3	1.1	—5.88	—281
nsf.	225									
5.1	908	1446	62.4	45						
nsf.	201				17.5	68	2.8	0.3	—0.34	—253
7.8	1030	915.7	44.8							
nsf.	204									
nsf.	214			45						
8.1	1143	489.6	26.6							
nsf.	[400]									
Grafting nsf.	482			55	78.1	19	17.8	1.6	—4.09	—567
4.5	1367	154.4	10							
Grafting. nsf.	Cardiac arrest. [400]			45					—1.04	—287
7.5	1438	40	3							
7.9	1129			20					—2.49	—290
nsf.	[200]									
Grafting nsf.	[400]			20					—2.61	—314
0.3	1294									
3.2	1053			60					—0.15	—198
monia 1.8	887			28					—1.79	—140

afting was done, and one pint of blood on the 25th post burn day resulting in cent take of grafts. — RCV was measured by the T-1824 technic. Tm was obtained according to method b) for the periods 5—19 days and 29—43 days (including value for day 47).

Curves 2 and 3 are seen to fall practically on top of each other up to day 29. From that day on curve 3 shows an increasing RCV, and curve 2 indicates a normal destruction. The picture resembles that of patient T. E. Destruction was found to be normal except for the period of operation where red cell loss was equivalent to about 1½ bottles of blood. During the first 14 days after infusion (coinciding with the temperature elevation) no production was found, resulting in a negative red cell balance. A similar pattern of red cell turnover and balance was found in other minor burns (Birkeland, 1958, d).

H. E. (Fig. 3, Table II). 58-years old colored male.

This patient represents a more severe burn, of back, head, neck and right arm — totalling 25 per cent (2° & 3°). RCV was measured by the P^{32}-technic. Blood was drawn for tagging 13 hours after burning.

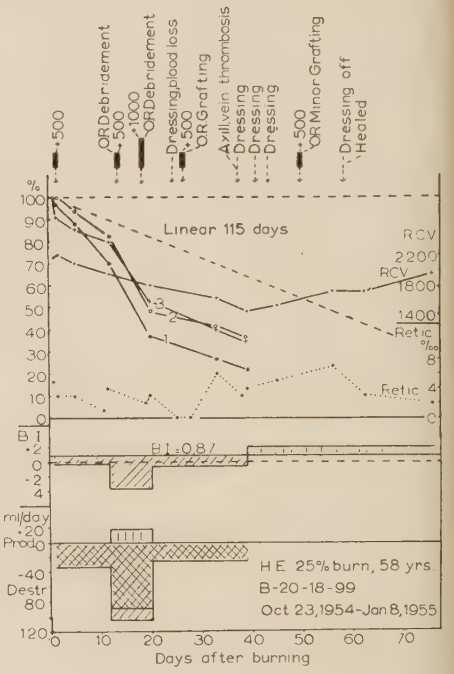

Fig. 3.

Curves 2 and 3 show essentially the same course throughout — indicating that curve 2 adequately describes the course of decline in RCV (when the effects of transfusions are eliminated). The lower values for curve 3 during days 2—12 indicate that untagged cells from the first transfusion were more rapidly destroyed than the tagged erythrocytes. In Table II the Tm = 111 days expresses the destruction rate of tagged cells, corresponding to 21.9 ml/day (in brackets). Total destruction of both tagged and untagged cells was found to be 34.7 ml/day.

The absence of production is noted during the first 39 days, except for the period from 12 to 20 days. However, this period is short (8 days) and also includes three transfusions of untagged cells so that the value for p duction is less accurate. — After day when most of the wound was covered w skin, the positive red cell balance is show

M. W. (Fig. 4, Table II). 39-years old white female.

This patient represents a very severe burn cause of the extent and depth of the full skin thi ness flame injury, including neck, chest, abdo and inguinal region. Also the patient suffered fr pulmonary tuberculosis and was under treatm with Streptomycin and PAS with a pneumo toneum. A history of chronic alcoholism was tained. — RCV was measured by the P^{32}-tech Cells were removed for tagging 3 hrs. 40 m following burning.

The marked early drop and relatively ra decline of the later part of curves 2 an

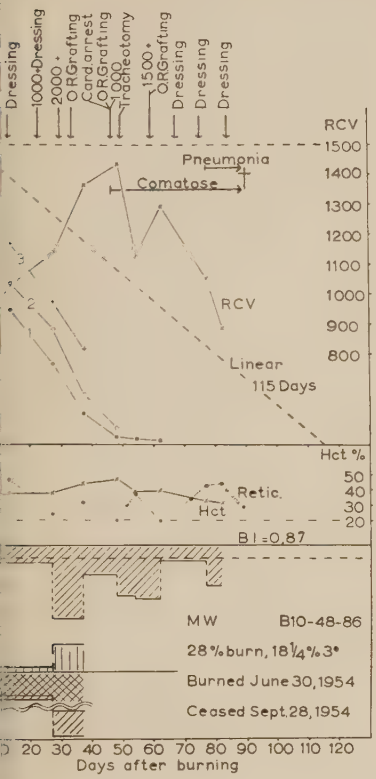

Fig. 4.

The difference in values for these day 5 is of the same order of as the early drop in Cr^{51}-survival n healthy individuals after infusion cells drawn from burn patients ter injury (Birkeland 1958, d). l indicate an initial selective loss cells that were already damaged by e time they were obtained from t for tagging. The higher level of compared to curve 2 during days ld be explained on such a basis. ems to be representative of the de- of total RCV during the latter

Comments

Difficulties arose when larger volumes of untagged cells were transfused during the period under analysis, particularly when operations also were being performed. The viable fraction of transfused cells estimated from storage time may be more or less accurate. Even though viable, they may show a different destruction rate from that of the rest of the circulating red cell mass. The Tm for tagged cells may be fairly accurately estimated even though an operation is being done. However, the loss of untagged transfused cells is also dependent on whether they were infused before, during or after the blood loss caused by operation. These limitations with respect to determination of destruction rate will be present regardless of the method used — unless cell survival is determined for each bottle of blood. Such a procedure was not found practical.

Total mass of untagged cells added to the circulation (B_t) is determined as the difference between red cell mass (RCV_t) at time t and the remaining portion (RCV_o') of the originally tagged red cell volume at time zero (RCV_o), cf. Fig. 1 (Birkeland, 1958 a). Consequently, B_t is influenced by the factors modifying destruction of cells given as transfusions. However, the red cell balance and B. I. directly describe the pattern of volume deficit, regardless of the underlying detailed mechanisms of turnover disturbance.

Of the four transfusions given to patient M. W. during days 27—37, two were given prior to and during operation and were assumed to be destroyed at the same rate as the Cr^{51}-tagged cells. Two bottles were administered after operation and were assumed to

be destroyed at a substantially normal rate. Patient M. W. appeared to afford the most uncertain evaluation of red cell turnover (particularly production) of all the burn patients studied.

Besides illustrating the limitations of the method, the diagram also pictures the relatively great changes in red cell turnover and balance that may be encountered in severe burns in relation to the clinical events.

DISCUSSION

The usefulness of the method presented may be judged on the basis of the validity of the assumptions and the practical applicability of the procedures involved. — Firstly, the elution rate over the first 50 days was assumed to be constant and equal to that observed in normals. Secondly, destruction rate of autoinfused Cr^{51}-tagged cells after the first day was assumed to be representative for all cells in circulation (Birkeland, 1958, a).

The first assumption could be tested directly only if destruction rate of autoinfused red cells were simultaneously tested by an independent and uniform tag of all cells, regardless of their age. Such a method is not available at present. Therefore, more indirect evidence must be looked for.

Red cells, drawn from severe burns within ten hours after injury, were infused into healthy volunteers (Birkeland, 1958 d). After the first 1—2 days they showed a Cr^{51}-survival within normal range in four out of five experiments. In the fifth experiment an increased slope was noted after the 16th post-transfusion day. — An increased rate of Cr^{51}-elution would, with the present method, yield falsely high values for production — and *vice versa*. From the patients presented as well as from others, also after infusion of fresh, normal Cr^{51}-tagged cells (Birkeland, 1958, d), the data gave no indication of increased elution after the occasional initial drop (cf. M. W.). A slower elution rate cannot be excluded. However, the low rate of production usually encountered in severe burns is consistent with the results obtained even in minor burns where increased destruction was unlikely (cf. T. J.).

From comparative studies with Cr^{51}-tagging and differential agglutination of heterotransfused cells in burn patients, Davies & Topley (1956) found similar changes in the transfused red cell volume by both methods. — By comparing survival as derived from Cr^{51}-data with those obtained by simultaneous differential agglutination, no obvious difference from the normal uniform rate of elution was found in hemolytic disorders (Hughes Jones & Mollison, 1956). — Assuming a uniform and normal rate of elution, red cell destruction has been determined in a variety of hemolytic anemias (Weinstein & LeRoy, 1953; Weinstein, Spurling, Katz & Necheles, 1954; Read, Wilson & Gardner, 1954; Donohue, Motulsky, Giblett, Pirzio-Biroli, Viranuvatti & Finch, 1955; Jones, McAlister & Prankerd, 1956). The results values were reported to be in good agreement with clinical and hematological findings as well as with Ashby data obtained in other patients of the same type. — Any positive evidence to show that the elution rate is obviously changed in pathological conditions is unknown to the present author.

From these considerations it is concluded that the first assumption most likely is a valid one for the present purpose.

ond assumption would not be valid nism were treating tagged and un- ls in a different way with respect tion. Such could be the case if the lls were suffering from an intra- efect that was not present to the ee in the untagged population, as ndicated in patient M. W. How- the occasional early rapid loss had e, tagged and untagged cells ap- be destroyed at the same average rne out by the diagrams presented. neous testing with another reliable ot feasible in the present study. In- healthy volunteers of cells that vn from burn patients after the e of injury (when infection was in- resent, often with antibiotic resi- ns) was deemed decidely inadvis-

more, as autoinfusion of cells was n 24 hours after their drawing, any in age distribution was negligible. sions may often be disturbing fac- scussed under patient M. W.

therefore be concluded that the sumption can only be valid as an ation to the true state. The obtained port the usefulness of this approxi-

tion of the method proved to be severely ill patients, during most states encountered and where f destruction and production were with time and clinical events. al turnover pattern varied with the injury and indicated a reasonable tion of the red cell balance changes observed.

The balance method, although laborious and time-consuming, was thought to be mandatory in order to correct for sampling and transfusions, both of which were of sufficient magnitude to influence the results considerably.

The method is thought to be of value also in patients of other types (cf. T. E.), particularly in surgical cases. — Following substantial disappearance of the originally infused Cr^{51}-tagged red cells, the analysis may of course be extended in time by repeated tagging.

SUMMARY AND CONCLUSION

The approach used for quantitative evaluation of red cell turnover was tested in patients with marked disturbances in red cell production, destruction or both.

The method was found to supply useful information for the purposes (see introduction) which lead to its development.

REFERENCES

Birkeland, S.: a) A method for quantitative evaluation of red cell turnover in humans. I. Normal values. Scandinav. J. Clin. & Lab. Investigation *10*, 72, 1958.

— b) A red cell balance index — B. I. Scandinav. J. Clin. & Lab. Investigation, 1958. In press. c) The use of the isotope Cr^{51} for studying destruction of red cells in healthy humans. Scandinav. J. Clin. & Lab. Investigation, 1958.

— d) A study in burns. In preparation.

Davies, J. W. L. & Topley, E.: The disappearance of red cells in patients with burns. Clin. Sci. *15*, 135, 1956.

Donohue, D. M., Motulsky, A. G., Giblett, E. R., Pirzio-Biroli, G., Viranuvatti, V. & Finch, C. A.: The use of chronium as a red cell tag. Brit. J. Haematol. *1*, 249, 1955.

Evans, E. I.: The early management of the severely burned patient. Surg., Gynecol. & Obst. *94*, 273, 1952.

Ham, T. H.: A syllabus of laboratory examinations in clinical diagnosis. Harvard University Press, Cambridge, Mass., U. S. A., 1952.

Hughes Jones, N. C. & Mollison, P. L.: The interpretation of measurements with Cr^{51}-labelled red cells. Clin. Sci. *15*, 207, 1956.

Joske, R. A., McAlister, J. M. & Prankerd, T. A. J.: Isotope investigations of red cell production and destruction in chronic renal disease. Clin. Sci. *15*, 511, 1956.

Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K. Jr., Hamilton, P. B. & Archibald, R. M.: Copper sulfate method for measuring specific gravities of whole blood and plasma. United States Navy Research Unit, Hospital of the Rockefeller Institute for Medical Research, 1943—44. The corrected line chart — see: Hawk, P. B., Oser, B. L. & Summerson, W. H.: Practical physiological chemistry. Blakiston, Philadelphia, ed. 12, 1947, pp. 553—556.

Read, R. C., Wilson, G. W. & Gardner, F. H.: The use of radioactive sodium chromate to evaluate the life span of the red blood cell in health and certain hematologic disorders. Am. J. Med. Sci. *228*, 40, 1954.

Weinstein, I. M. & LeRoy, G. V.: Radioactive sodium chromate for the study of survival of red blood cells. — II. The rate of hemolysis in certain hemolytic disorders. J. Lab. & Clin. Med. *47*, 368, 1953.

Weinstein, I. M., Spurling, C. L., Klein, H. & Necheles, T. F.: III. The abnormal hemoglobin syndromes. Blood *9*, 1155, 1954.

ndinav. J. Clin. & Lab. Investigation 91—96, 10, 1958.

THE FIBRINOLYTIC AGENTS IN SALINE EXTRACTS OF HUMAN TISSUES

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(Received for publication November 13, 1957)

The existence of an activator of plasmino- in different human and animal tissues s first demonstrated by Astrup & Permin 947). This activator is fairly thermostable, ecially at acid reaction, (Astrup & Stern- ff, 1956), and it differs from a labile ivator of plasminogen which can be formed blood (Müllertz, 1955).

The physiological significance of the tissue ivator has been discussed for some years. tivation of the plasminogen normally pre- t in the blood can only occur if contact established between intravascular and in- cellular space. However, the tissue acti- or is bound firmly to the structural cell oteins and attempts by previous workers extract the active components and obtain m in a soluble form were unsuccessful leischer & Loeb, 1915; Permin, 1947). e insolubility of the plasminogen activator the tissues led to the suggestion that this ivator was chiefly of local importance strup, 1954, 1956).

Astrup & Stage in 1952 succeeded in ex- cting the activator from the tissues with SCN, which made possible a quantitative imation of the activator (Astrup & Al- chtsen, 1957). This method is of no help the understanding of its immediate avail- lity to the organism, since it is based on unphysiological procedure.

Saline extracts of tissues show fibrinolytic activity (Macfarlane & Biggs, 1948). Since the active agents in such extracts probably reflect the activity immediately available to the organism, we have studied the composition of these solutions. The present paper demonstrates the existence of two different plasminogen activators in saline extracts from human tissues.

MATERIALS AND METHODS

Samples of human tissues were obtained at autopsy. They were investigated on the same day or left at —20° C after superficial cleaning with tap water. Only apparently normal tissues were analysed.

The fibrinolytic activity was estimated by the fibrin plate method of Astrup & Müllertz (1952), which estimates the content of plasmin as well as of plasminogen activators. By heating the fibrin plates for 35 minutes at 85°, the plasminogen in the fibrin plates is destroyed (Lassen, 1952) and the substrate reacts with plasmin only. Dilution curves were prepared by applying different concentrations of the tissue extracts to the fibrin plates. The fibrinolytic activity was recorded as the diameter product of the lysed zones (in sq. mm) after 20 hours digestion at 37° (average of three determinations of each dilution).

The buffer was diethylbarbiturate buffer (Michaelis), pH 7.8, 0.05—M, containing 0.1—M NaCl. Total $\mu = 0.15$. Thrombin for the fibrin plates was kindly supplied by the Løvens Kemiske Fabrik, Copenhagen.

RESULTS

Activity of the extracts:

The tissue samples were treated in a Potter homogenizer with 5 ml of 0.9 per cent NaCl per gram tissue and then shaken for 1 hour. After centrifugation for 10 minutes at 19000 r. p. m. the supernatant was collected and 5 ml of 2-M KSCN was added to the sediment. After shaking for 1 hour the tissue was again removed by centrifugation and the activities of the salt water extract and of the KSCN extract estimated.

No activity was recorded when any of the saline or KSCN extracts were applied to the heated fibrin plates. Therefore the activity is not caused by fibrinolytic enzymes but by activators of plasminogen.

The results of the estimations on normal plates appear in Table I.

All saline extracts from the thyroid gland, the kidney, the uterus, the ovary and the lymphatic glands were active. Activity was also found in some of the samples from lung, adrenal gland, muscles, brain and heart whereas all saline extract from liver, spleen and testes were inactive. It is obvious that the amount of plasminogen activator in the saline extracts differs considerably from one tissue to another.

When the KSCN extracts were applied to the standard fibrin plates, activity was observed in all cases but two (liver and spleen). The activity was always higher than in the corresponding saline extract (Table I). This means that only a small fraction of the total activity is present in the salt water and that more can be extracted with KSCN, which is in accordance with our previous findings. In a few cases the saline extract approached the KSCN extract in activity.

Heat stability of saline extract:

In order to characterize the activating components in the saline extracts the stability at different temperatures and pH values was investigated in the following manner: The pH was regulated to different values (with 0.1-n HCl, 0.1-n NaOH and various buffers (citrate, phosphate and borate)) and the samples heated for 30 minutes at 37°, 50° 70°. After cooling to 0° in ice water and neutralizing, they were applied to the plates in order to estimate the remaining activity (typical examples in Fig. 1). The activating components were most stable at pH values around neutral reaction at 37° and 50°. At 70° the activating compounds were most stable at acid reaction and completely destroyed at neutral and alkaline reaction. These curves differ from those obtained with the stable tissue activator of plasminogen (Astrup & Sterndorff, 1956, Albrechtsen, 1956) which is stable at acid reaction at 37° and 50°. However, the stability at 70° is similar to the stability of the stable activator. The curves differ also from those ob-

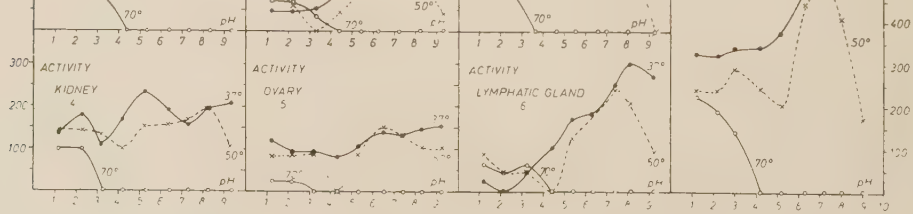

Fig. 1. Heat stability of saline extracts from human tissues after heating for 30 minutes. *Abcissa:* pH of the samples during heating. *Ordinate:* Activity recorded as a diameter product (average of three determinations).

for the plasminogen activator in the blood ertz, 1955) which is completely destroyed at reaction (especially at 70°). The remaining ty at 70° at acid reaction combined with the activity at 37° and 50° at more alkaline re- indicate that the saline extract contains a as well as a labile activator of plasminogen.

Heat stability of KSCN extract:

nce the difference observed between the sta- s of the activating components in the saline ct and in the KSCN extract could be caused ntaminating proteins, the following experi- were carried out: A salt water extract and CN extract were prepared from human lung scribed above. The KSCN extract was pre- ted with 1-n HCl at pH 1 after diluting 8 with distilled water. After centrifugation the itate was redissolved in the original volume rbital buffer. 5 ml of the saline extract and of the precipitated KSCN extract were heated ° for 30 minutes to destroy all activity. 5 ml e original saline extract was then mixed with eated KSCN extract and 5 ml of the un- d KSCN extract mixed with the heated saline ct. The solutions so obtained contain similar nts of proteins and salts, and it is therefore ble to compare their stability curves directly. stabilities at different temperatures and pH s were estimated as described (Fig. 2). The lity of the mixture of the saline extract with eated KSCN extract corresponds to that ob- d with the original saline extract, and the lity of the mixture of the KSCN extract with eated saline extract corresponds to that of the e tissue actvitaor of plasminogen. These results rm the conclusion that the activating com- ds in saline extracts differ from the stable e activator of plasminogen.

eparation of the activating compounds in saline extracts

ie activity remaining at acid reaction in the e extracts (especially at 70°) indicates the pre- of small amounts of the stable tissue acti- in these solutions. This was confirmed as ws:

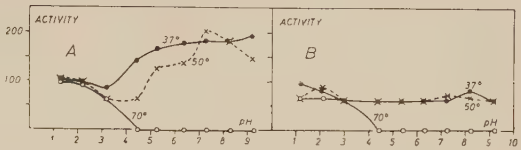

Fig. 2. Heat stability of a saline extract (A) and a KSCN extract (B) from the human lung (for details see text). *Abscissa* and *Ordinate:* see Fig. 1.

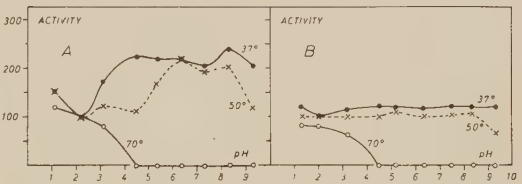

Fig. 3. Heat stability of saline extract from the human uterus before (A) and after (B) heating for 30 minutes at 37° and pH 3.0. *Abscissa and Ordinate:* see Fig. 1.

I. A saline extract prepared from human uterus was divided into two portions. In the first portion the activator stability was estimated as described. The second portion was adjusted to pH 3.0 with 1-n HCl and heated for 30 minutes at 37°. After neutralization the stability of the remaining activity was estimated (Fig. 3). The stability curve obtained after heating corresponds to the curve obtained for the stable tissue activator of plasminogen and differs from that obtained with the original extract. These results indicate that a labile component in the salt water extract is destroyed by heating at pH 3.0°, while a stable activator remains in the solution. The results confirm the conclusion drawn that the saline extract contains two different activators of plasminogen.

II. Saline and KSCN extracts were prepared from human thyroid gland as described above. The KSCN extract was precipitated with 1-n HCl and redissolved in barbital buffer as described. The activities of the saline extract, of the precipitated KSCN extract and of a mixture of equal amounts of these two extracts were estimated and the acidity of each of these three solutions adjusted to pH 3 with 1-n HCl. The solutions were placed in a water bath at 37° and samples were removed every 5

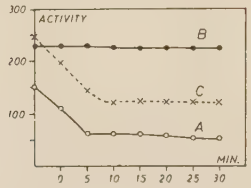

Fig. 4. Heat stability of saline extract (A) and a precipitated KSCN extract (B) and a mixture of these (C) after varying periods of time at 37° and pH 3 (human thyroid gland). *Abscissa:* Heating time in minutes. *Ordinate:* Activity.

minutes, neutralized, cooled to 0° in ice water and applied to the standard plates in order to estimate the activity remaining (Fig. 4). The activity of the precipitated KSCN extract was constant during the period of heating whereas the activity of the saline extract decreased to a certain level within the first 5 minutes and then became stable. The curve for the mixed solution was similar to the curve for the saline extract alone but the stable level had a higher activity corresponding to a higher concentration of the stable activator in this mixture. These results demonstrate the existence in the saline extract of a labile compound which is destroyed after 5 minutes heating at pH 3. The activity recorded after 5 minutes heating is caused by the existence of a stable compound remaining in the extract. Similar compounds are found in the mixed solution, but the precipitated KSCN extract contains only the stable activator.

III. This was further substantiated by the following experiment: Saline and KSCN extracts were prepared from the thyroid gland as described. The KSCN extract was precipitated as described

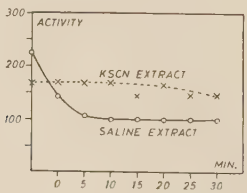

Fig. 5. Heat stability of mixtures of extracts of human thyroid gland (for details see text). *Abscissa and Ordinate:* see Fig. 4.

and the precipitate redissolved in the original volume of barbital buffer. 2.5 ml of the saline solution and 2.5 ml of the precipitated KSCN solution were heated to 70° for 30 minutes, cooled and mixed with 2.5 ml of the original precipitated KSCN solution and the original saline extract respectively. Samples were removed for activity estimations. The acidities were then adjusted to pH 3 with 1-n HCl and the solution placed in a water bath at 37°. Samples were withdrawn every 5 minutes, neutralized, and the activities estimated (Fig. 5). The curves are similar to those recorded in Fig. 4 indicating that labile and stable activators are present in the saline extract but only a stable activator in the precipitated KSCN extract.

DISCUSSION

Macfarlane & Biggs (1948) demonstrated fibrinolytic agents in saline extracts from different human tissues, especially the human lung. Fantl & Fitzpatrick (1950) found an activator of plasminogen in saline extracts of the human brain and they suggested that the ease with which the brain yields the activator (as contrasted to other organs) was possibly due to a solubilizing effect of the phospolipids. Astrup & Sterndorff (1952) found an activator of plasminogen in saline extracts from the human lung (and also in pig brain). These results and those here presented might be of aid in the understanding of the significance of the fibrinolytic activity in tissues. A saline extraction is closer to a physiological process than extraction with KSCN. The existence of a labile activator of plasminogen (corresponding to the activator found in the blood) in saline extracts from certain human tissues makes it conceivable that this activator could be released into the blood stream under certain conditions, thus activating the plasminogen in the plasma.

ble I: *Fibrinolytic activity of saline ucts of human tissues compared with the ity of the KSN extract of the insoluble ue remaining after the saline extraction. ity recorded as diameter product (mm²) of the stock solution.*

e	Saline extract	KSCN extract
	0, 0, 182, 210, 529	110, 144, 306, 525, 576
oid	108, 120, 625	266, 400, 835
d		
t	0, 0, 0, 0, 196	156, 225, 235, 270, 400
n	81, 0, 105	169, 195, 205
	0, 0, 0	0, 55, 142
n	0, 0, 0, 0	0, 100, 110, 144
ey	80, 81, 140	225, 266, 370
s	0, 0, 0, 0	120, 150, 156, 200
s	306, 626	529, 961
y	448	575
les	64, 0, 136, 0	144, 190, 284, 324
hatic	245, 256, 361	336, 535, 576
d		
nal	0, 120, 164, 400	155, 196, 540, 770

he relationship between the stable and abile activators in the tissues is unknown. presence demonstrated here of large unts of labile activator in saline extracts tissues known to contain large amounts he stable activator (Albrechtsen, 1957) s further investigations. This applies to the great variability in the concentra- of available activator in the saline ex- s of the same organ as demonstrated in le I. The degree of autolysis in the tissues stigated could be of importance for this ability by making the activator more or extractable.

here is reason to believe that the activity he saline extracts reflects the immedi- availability of the plasminogen activator he organism.

mall amounts of blood may persist in the es and thus furnish an activator of the e type. The moderate concentration of this activator in *post mortem* blood (Müllertz, 1953) combined with the high dilution of these components in the tissue extracts make it improbable that the labile activator in the saline extracts originates solely from the blood present in the organs.

SUMMARY

1. Saline extracts from different human organs (thyroid gland, kidney, uterus, ovary, lymphatic glands, lung, adrenal, muscles, brain, heart) contain fibrinolytic activity.

2. This is caused by two different activators of plasminogen.

3. One activator is labile and corresponds to the plasminogen activator in the blood, the other is stable and corresponds to the stable tissue activator.

4. There is reason to believe that the activity in the saline extracts reflects the immediate availability of the plasminogen activator for the organism.

ACKNOWLEDGEMENTS

This investigation was aided by grants (to Dr. Tage Astrup, Biological Institute of the Carlsberg Foundation, Copenhagen) from the Josiah Macy, Jr. Foundation, New York, and from the National Danish Association against Rheumatic Diseases. Thanks are due to Miss Mona Rimestad for technical assistance.

REFERENCES

Albrechtsen, O. K.: The fibrinolytic activity of the human endometrium. Acta Endocrinol. *23,* 207, 1956.

— The fibrinolytic activity of human tissues. Brit. J. Haematol. *3,* 284, 1957.

Astrup, T.: Fibrinolysis and thrombosis. In: "Thrombosis and Embolism". Benno Schwabe and Co., Basel 1954, p. 92.

— Fibrinolysis in the organism. Blood, *11,* 781, 1956.

Astrup, T. & Albrechtsen, O. K.: Estimation of the plasminogen activator and the trypsin inhibitor in animal and human tissues. Scandinav. J. Clin. & Lab. Investigation *9,* 233, 1957.

— & Müllertz, S.: The fibrin plate method for estimating fibrinolytic activity. Archiv. Bioch. Bioph. *40,* 346, 1952.

— & Permin, P. M.: Fibrinolysis in the animal organism. Nature *159,* 681, 1947.

— & Stage, A.: Isolation of a soluble fibrinolytic activator from animal tissues. Nature *170,* 929, 1952.

— & Sterndorff, I.: Fibrinolytic activity of tissue extracts and of trypsin. Nature *170,* 981, 1952.

— — The Plasminogen activator in animal tissues. Acta Physiol. Scandinav. *36,* 250, 1956.

Fantl, P. & Fitzpatrick, M.: Fibrinolysis induced by brain extracts. Brit. J. Exper. Pathol. *31* 131, 1950.

Fleisher, M. S. & Loeb, L.: On tissue fibrinolysin J. Biol. Chem. *21,* 477, 1915.

Lassen, M.: Heat denaturation of plasminogen i the fibrin plate method. Acta Physiol. Scan dinav. *27,* 371, 1952.

Macfarlane, R. G. & Biggs, R.: Fibrinolysis. It mechanism and significance. Blood *3,* 116 1948.

Müllertz, S.: A plasminogen activator in spon taneous active human blood. Proc. Soc. Exper Biol. Med. *82,* 291, 1953.

— Formation and properties of the activator o plasminogen and of human and bovine plasmi Biochem. J. *61,* 424, 1955.

Permin, P. M.: Properties of the fibrinokinase fibrinolysin system. Nature *160,* 571, 1947.

dinav. J. Clin. & Lab. Investigation 97—101, 10, 1958.

QUANTITATIVE ESTIMATION OF CEREBROSIDES IN PLASMA

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(Received for publication October 21, 1957)

he occurrence of glycolipids in blood sma and their physiological importance e been subject to a few investigations . In 1938 Kirk (1938 a) reported figures the content of cerebrosides in normal sma. The cerebroside level varied between nd 167 mg/100 ml in 13 normal cases. a material of 14 patients with pernicious mia the variations were greater still irk, 1938 b). Erickson, Souders, Sherd, Teague & Williams (1940) found a siderably lower normal value for cerebroes, 15 mg/100 ml; the individual figures re not reported, however.

hese investigators determined the cereside content in plasma by analysing the ucing power in a lipid extract before and r hydrolysis. The increase in the reducing ver after hydrolysis was suggested to be to sugar, released from cerebrosides. As reducing methods are unspecific, they are uitable for the determination of low contrations of cerebrosides, which is obvious . from the large variations found by Kirk 938 a, 1938 b). A lot of substances of a molecular weight are extracted together h the lipids (Folch & Van Slyke, 1939) it is unknown, whether their reducing ver is unchanged after hydrolysis. Lipids er than cerebrosides containing reducing substances, *e. g.* gangliosides, also contribute to the reducing value.

In order to study the cerebroside level in certain hematological and reticuloendothelial diseases, it was thus necessary to elaborate a more specific method for quantitative estimation of cerebrosides. We have applied a modification of the method adopted by one of us (Svennerholm, 1956) for the estimation of cerebrosides in nervous tissue. As the lipid extract was also to be used for the determination of other lipid components in plasma, the extraction procedure was made quantitative for all the lipids.

EXPERIMENTAL

Reagents and apparatus

Orcinol reagent. 100 ml of concentrated sulphuric acid was added under cooling in running water to 4 ml of a 5 per cent orcinol solution in water. The reagent was made at least 15 hours before use to reduce the blank color. In other respects the reagents and apparatus of the previous study were used (Svennerholm, 1956).

All glass-ware and needles used for the sampling of blood were siliconized.

Method

Plasma sampling. The blood samples were drawn from the subjects in the morning — after a fast of approximately 10 hours — into centrifuge tubes containing 5 ml of 3 per cent sodium citrate to a

final volume of 50 ml. At the same time the hematocrit value was determined in order to allow calculation of the plasma dilution. The blood was centrifuged immediately at 1500 r. p. m. for 30 minutes at + 4° C. The plasma was separated and recentrifuged for 30 minutes, now at 3000 r. p. m. to remove remaining cells. 10 ml samples of plasma were pipetted in 250 ml ground flasks, 5 ml of 0.2 M phosphate buffer, pH 6.0, was added and the content was lyophilized. (The addition of phosphate buffer can be omitted, when only cerebrosides are determined.)

Lipid extraction. The lyophilized plasma was boiled under reflux with 50 ml of chloroform-methanol (2 : 1 v/v) for 30 minutes. The extract was filtered into a clean, ground flask and the protein precipitate rinsed three times with 10 ml of the extraction solvent. The solvent was evaporated *in vacuo* and the residue dissolved in 10 ml of the solvent mixture and filtered into a graduated cylinder of 25 ml equipped with a ground glass stopper. The flask was rinsed three times with 2 ml of the solvent and, finally, the volume adjusted exactly to 20 ml with chloroform-methanol. 5 ml of 0.1 per cent sodium chloride solution was added. The glass stopper was moistened with distilled water and the cylinder shaken vigorously for one minute and then left for 48 hours. Two phases then appeared. The upper phase was removed with a ballooned pipet and discarded. The cylinder wall was rinsed three times with 2 ml of the upper phase from a mixture of 800 ml chloroform-methanol (2 : 1 v/v) and 200 ml of 0.1 per cent sodium chloride, the rinse solution being removed after each washing. The content of the cylinder was then transferred to a large Thunberg tube and the solvent evaporated in a stream of air at 40° C. If other lipid components also were analysed, the volume was made up to 25 ml with chloroform-methanol (2 : 1 v/v) and a aliquot of 20 ml was used for the determination of cerebrosides.

Hydrolysis. The lipids were dissolved in 0.5 ml of ethanol and 2 ml of 3 N sulphuric acid was added. Hydrolysis was performed for two hours in a boiling water bath. After cooling to room temperature the hydrolysate was filtered through an acid-washed filter paper into a 10 ml cylinder. The hydrolysis tube and the filter were rinsed with 3 N sulphuric acid and finally the volume was adjusted to 10 ml with the acid.

Color reaction. It was performed as described in the original method (Svennerholm, 1956).

COMMENTS ON THE METHOD

1. The method was adopted for plasma instead of serum as a release of lipids may occur from the blood cells during the coagulation. Sodium citrate is chosen as an anticoagulant, as heparin ought to be avoided; it might form water-soluble complexes with the lipids, *e. g.* free fatty acids and phospholipids. It was also observed that the separation of the two phases was delayed by heparin.

2. To achieve a quantitative extraction of all the lipids it is necessary to use a strong polar solvent as, otherwise, a part of the free fatty acids and phospholipids is not released from the proteins. The common Bloor extraction does not give a quantitative extraction of the glycolipids in nervous tissue (Brante & Svennerholm, unpublished results). Methanol-chloroform mixtures, however, are suitable for the extraction of glycolipids and in combination with partition technique the extract can also be practically freed from non-lipid material. The plasma is lyophilized before the extraction to reduce the solvent volume.

3. In the partition procedure the lipids including the cerebrosides are distributed in the lower (chloroform) phase and the non-lipid material in the upper (water) phase. Gangliosides and a group of closely related glycolipids also occur in the upper phase (Svennerholm, 1956; unpublished results).

To prove that the chloroform phase of

ble I. *Recovery of added cerebrosides to lipid extracts of plasma*

rosides lasma ng	Cerebrosides added mg	Cerebrosides found mg	Recovery per cent
.357 (4)[1]	0.524	0.908	105
.357 (4)[1]	1.048	1.437	103
.474 (5)[1]	0.731	1.207	100
.474 (5)[1]	1.462	1.840	93

[1] Number of analyses.

na lipid extract contains cerebrosides, the tion procedure was applied on samples liters of plasma. The chloroform phase freed from phospholipids with magne- trisilicate. Cholesterol, cholesterol esters glycerides were removed by repeated ipitations with acetone. In other experi- ts the procedure of Klenk & Leupold 44) was used to eliminate the phospho- s. The yield of purified material, calcu- l from the sugar analysis, was about 50 cent. The ratio nitrogen/hexose was 1.1. aper partition chromatography, galactose identified on the hydrolysed material.

he partition of the glycolipids between two phases was tested by adding prepara- s of brain cerebrosides and gangliosides pid extracts of plasma. The recovery of ed cerebrosides was from 95—105 per (Table I). An addition of 0.5 and 1.0 of gangliosides to 10 ml of plasma did give any significant change of the cere- ide content. However, the standard error ne method is so large (see below) that or differences cannot be detected.

o avoid the passage of fatty acids in the n of soaps to the water phase, the pH of plasma is lowered by adding phosphate buffer before lyophilization. The lower phase is then suitable for the analyses of all lipids in plasma, except gangliosides and related glycolipids. At the analysis of total lipids, the loss of the gangliosides will give a negative error, but this is negligible as they constitute only about 3 mg/100 ml.

4. In the original method distilled water was used to rinse the hydrolysis tubes and the filters. When this procedure was applied the filtrates became opalescent because of the large amount of lipids in the hydrolysates. (The ratio cerebrosides/total lipids is much lower in plasma than in nervous tissue.) By rinsing with 3 N sulphuric acid instead of water no opalescence occurred. The sulphuric acid concentration of the orcinol reagent was then reduced correspondingly to achieve optimal acidity in the color reaction.

5. The accuracy of the method was estimated from 16 consecutive determinations of the cerebroside content in plasma. The mean value was 4.31 mg/100 ml and the standard deviation $\pm$ 0.421 mg/100 ml, which means a standard error of $\pm$ 9.8 per cent. This error includes errors originating from the extraction, the partition, the hydrolysis and the color reaction. The two latter steps give the largest error, as the standard deviation was $\pm$ 7 per cent when 10 samples of the same lipid extract were hydrolysed and the amount of cerebrosides determined. The standard error reported here is considerably larger than that earlier reported for nervous tissue (Svennerholm, 1956). The concentration of cerebrosides in relation to other lipids is, however, many fold lower in serum than in nervous tissue. Also the total amount of cerebrosides is too low for more accurate analyses.

APPLICATION OF THE METHOD

Normal values were determined in plasma from 25 persons, 21 men and 4 women, aged 28—82, the majority being between 50—70 years. The subjects were hospitalized in the Medical Clinic II, Sahlgren's hospital. All had normal blood values and no other signs of hematological disease. The mean value was 4.36 ± 0.18 mg per 100 ml.

The values found in this investigation are many fold lower than those previously reported (Kirk 1938 a; Erickson, Souders, Shepherd, Teague & Williams, 1940). The discrepancy may partly be explained by the existence of water-soluble glycolipids in plasma. In many other sources there are glycolipids which contain more carbohydrates than do the cerebrosides. Klenk & Lauenstein (1953) have shown the existence of glycolipids other than cerebrosides in the red blood cells and the spleen. The best known of these are gangliosides (Fig. 1). But there are also intermediate forms between cerebrosides and gangliosides which contain hexoses and hexosamine or only hexoses.

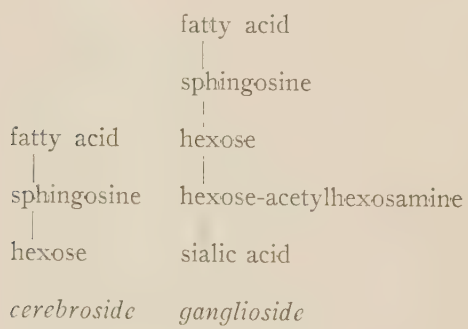

Fig. 1.

Because of their large content of carbohydrates they are more or less water-soluble, *e. g.* gangliosides are quantitatively partitioned in the upper phase in the partition procedure (Svennerholm, 1956).

In human plasma we found a ganglioside content of 2.5 mg per 100 ml (Svennerholm & Svennerholm, 1956). As the reducing value of gangliosides is twice that of cerebrosides this amount corresponds to 5 mg per 100 ml. It is probable that there are also other water-soluble glycolipids, even though we have not been able to show their existence.

Besides the glycolipids, non-lipid bound reducing substances are extracted with organic solvents. This observation was made already by Kimmelstiel (1929). Therefore he determined the reducing ability of the lipid extract before and after hydrolysis, and considered the difference as lipid-bound sugar. He did not account for any increase in the non-lipid reducing substances after hydrolysis. This is contradictory to our findings. The upper phases from several samples of plasma lipid extracts were combined and evaporated *in vacuo* to dryness. The glycolipids were extracted with dry chloroform-methanol (2 : 1 v/v). On the residue of non-lipid material the reducing ability was 10—25 per cent greater after hydrolysis than before. It is therefore probable that non-lipid material have contributed to the values of cerebrosides in earlier investigations.

SUMMARY

A method for the quantitative estimation of "true" cerebrosides in plasma is described and discussed. The extraction procedure of the cerebrosides applied is also convenient for the analysis of the other lipids in plasma.

he mean value of cerebrosides in plasma determined to 4.36 ± 0.18 mg per 100 This value is many fold lower than the res previously reported, the reasons for ch are discussed.

ACKNOWLEDGEMENT

he expenses were partly defrayed by a grant E. Svennerholm from the Medical Faculty, versity of Gothenburg. The technical assistance Miss Sybil Rohman is gratefully acknowledged.

REFERENCES

nte, G. & Svennerholm, L.: Unpublished data.
kson, B. N., Souders, H. J., Shepherd, M. L., Teague, D. M. & Williams, H. H.: Relation between cerebroside and neutral fat content of blood plasma, erythrocytes and stroma. Proc. Soc. Exper. Biol. & Med. *45,* 153, 1940.
ch, J. & Van Slyke, D. D.: Nitrogenous contaminants in petroleum ether extracts of plasma lipids. J. Biol. Chem. *129,* 539, 1939.
Kimmelstiel, P.: Fortgesetze Untersuchungen über die Cerebroside. Biochem. Ztschr. *212,* 359, 1929.
Kirk, E.: The concentration of lecithin, cephalin, ether-insoluble phosphatide and cerebrosides in plasma and red blood cells of normal adults. J. Biol. Chem. *123,* 637, 1938 a.
— The concentration of the individual phosphatides and of cerebrosides in plasma and red blood cells in pernicious anemia before and during liver treatment. J. Am. Med. Sci. *196,* 648, 1938 b.
Klenk, E. & Lauenstein, K.: Über die Glykolipide und Sphingomyeline des Stromas der Pferdeerythrocyten. Ztschr. f. physiol. Chem. *295,* 164, 1953.
— & Leupold, F.: Über eine vereinfachte Methode zur Darstellung von phosphorfreien Cerebrosiden. Ztschr. f. physiol. Chem. *281,* 208, 1944.
Svennerholm, E. & Svennerholm, L.: Determination of glycolipids in plasma. Acta Chem. Scandinav. *10,* 1048, 1956.
Svennerholm, L.: The quantitative estimation of cerebrosides in nervous tissue. J. Neurochem. *1,* 42, 1956.
— Unpublished data.

Scandinav. J. Clin. & Lab. Investigation 102—107, 10, 1958.

IRON DEFICIENCY AND TREATMENT IN BLOOD DONORS STUDIED BY THE IRON ABSORPTION TEST

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(Received for publication November 9, 1957)

The purpose of this investigation was to determine whether it is desirable to give additional iron to regular blood donors in this institution. With this in mind, an attempt was made to establish whether they could be shown to suffer from any iron dificiency, and if so, how early in their careers as blood donors the sideropenia could be demonstrated, and to what extent it could be cured by the administration of iron by mouth. In addition to this an attempt was made to evaluate relatively the fasting serum iron determination and the iron absorption test as criteria of iron deficiency.

From theoretical calculations of iron intake and loss in blood donors, it appeared probable that sideropenia would gradually develop. A male who is not a blood donor normally loses or excretes a total of 0.5 to 1.5 mg of iron per day (Moore & Dubach, 1956; Sturgis, 1955; Whitby & Britton, 1953), and this must be replaced by absorption of the same quantity from the diet. An average day's diet contains approximately 12—15 mg of iron, of which it is calculated that the individual can absorb 5—15 per cent, that is 0.6—2.25 mg (Moore & Dubach, 1956; Sturgis, 1955). A male will therefore usually replace his physiological iron loss from his normal diet. A woman, however, loses 10—40 mg of iron each month during menstruation, an average of approximately 1 mg a day, so that her total iron requirements are about 2 mg daily. The iron balance therefore, in human males provides a narrow margin on the positive side, whereas in women the balance will often be negative.

A blood donor who, as in this institution, gives 450 ml every three months will lose an additional quantity of iron amounting to 2.5 mg per day. Donors' iron requirements are raised, therefore, in men to approximately 3.5 mg per day and in women to approximately 4.5 mg per day, which is in both cases more than they can be expected to absorb from a normal diet. Such a donor will, according to these calculations, develop an iron deficiency, in the case of a female in the reproductive period of life to the extent of 225 mg in 3 months, in the case of a male, of 135 mg. These deficits must be covered by the administration of additional iron by mouth.

In a number of recently published investigations (Bateman, 1951; Bergmann & Vogl, 1956; Goldeck & Remy, 1952; Goldeck & Remy, 1953; Kölwel, 1952; Möller, 1952; Möller & Jürgens, 1952; Remy, Goldeck & Krüger, 1952) it has been shown that

sideropenia which was expected from e theoretical considerations did in fact lop. Iron deficiency has been demon- ted even in blood donors who have given nore than 1300 ml per year (Holländer tampfli, 1956). In the earlier stages such ropenia is only demonstrable by means he iron absorption test, and not until it uch further advanced can it be expected eveal itself by a significantly low serum concentration. The hemoglobin concen- ion in the blood will, as a rule, only begin lrop when the body's iron depots are pletely or almost completely exhausted eilmeyer & Begemann, 1951; Jasinski, 9; Jasinski & Roth, 1954; Roth, 1950; ngle, 1957).

recognition of these facts the hemoglobin e alone should not be relied upon to rmine the suitability of a person as a d donor (Wachsmuth, Lutzeyer & Hein- , 1953), and the necessity of giving extra even to a donor with a normal hemo- in value should be realised (Bergmann ogl, 1956; Holländer & Stampfli, 1956; y & al. 1952; Remy & Zöckler, 1956; ty, 1941). Matthes & Kramer (1956) e shown that even with the addition of g of iron during the period between ations, in the form of iron combined with rbic acid, sideropenia can result from the ations in cases where 400 ml or more are n at intervals of 6—8 weeks.

METHODS

the present investigation the hemoglobin de- inations were performed with a Sicca hemo- r, standardized by comparison with the cyan- emoglobin method, and hematocrit determina- with Wintrobe hematocrit tubes which were ifuged for 45 minutes at 3500 r. p. m. Serum iron determinations were performed as described by Bröchner-Mortensen & Olsen (1940), and iron absorption tests as described by Jasinski & Roth (1954) with 176 mg of iron in the form of 1.6 g of ferrous gluconate (8 tabl. Ferronicum Sandoz) by mouth. Each test began at 8.30 a. m. when the sample for determination of the fasting serum iron concentration was taken. Immediately thereafter the subjcet was given the test dose of iron. The subsequent blood samples were taken at 9.30 a. m., 11.30 a. m. and 3.30 p. m. After withdrawel of the 9.30 a. m. sample the subject was allowed to eat a normal meal.

Iron treatment, when given, was always in the form of a course of 100 tablets ferrosi chloridum 0.267 g (Tabl. Ferrali forte "Nyco") over a period of 17 days. The iron absorption tests, when performed after a course of iron treatment, were performed at least 5 days after the conclusion of the treatment period.

RESULTS

As a preliminary, iron absorption tests were performed on 9 blood donors who had given from 1000 to 3150 ml blood in the past 2 years, and on 5 donors before their first donation (normal controls). The iron absorption curves for these 14 individuals are shown in Fig. 1, and some other hematological observations in the same subjects appear in

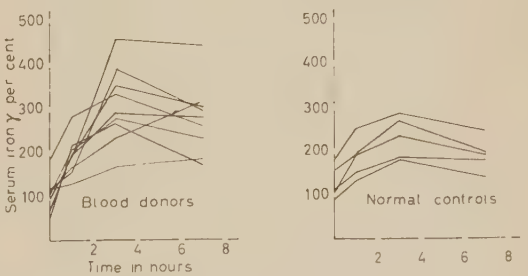

Fig. 1. Iron absorption tests on 9 blood donors who had given from 1000 to 3150 ml in the past 2 years and on 5 donors before their first donation (normal controls). For additional observations see Table I.

Table I. *Hematological observations in blood donors and controls.*

Name	Sex	Age in years	Ml donated past 2 years	Red cell count (millions per cmm)	Hemoglobin (gm per 100 ml)	Mean corpuscular hemoglobin (γγ)	Serum iron concentration during test (γ per 100 ml)		
							Fasting I	Maximal observed II	Difference II—I
H. H.	M	35	2,700	5.22	15.0	28.7	185	334	149
R. B.	F	42	2,700	4.81	13.5	28.1	120	188	68
E. T.	M	47	2,700	4.84	14.4	29.8	103	280	177
L. G.	F	34	3,150	4.38	12.8	29.2	115	322	207
P. M.	F	33	2,000	4.59	12.7	27.7	120	290	170
B. W. J.	M	40	3,150	4.44	12.4	27.9	96	460	364
A. W.	F	28	3,150	4.97	13.1	26.4	72	352	280
G. S.	F	33	2,250	4.62	12.8	27.7	72	292	220
I. B.	F	40	1,000	4.40	12.2	27.7	50	266	216
I. P.	F	23	0	4.94	13.6	27.5	90	182	92
I. W. K.	M	28	0	5.51	14.9	27.0	176	286	110
J. E.	M	27	0	4.66	13.4	28.8	158	238	80
O. P. F.	M	39	0	5.29	14.5	27.4	112	188	76
E. L.	M	26	0	4.90	14.5	29.6	103	272	169

Table I. With 3 exceptions (I. B., B. W. J. and J. E.) the hemoglobin values were above the permitted minimum for blood donors in this institution, namely 13.8 g/100 ml for men and 12.4 g/100 ml for women. Of these 3, 2 were donors and 1 a normal control.

The fasting serum iron concentration of the blood donors varied between 50 and 185 γ per cent with an average of 104 γ per cent. The fasting serum iron concentration of the normal controls varied between 90 and 176 γ per cent with an average of 128 γ per cent. There was no significant difference between the observed values in these two groups when tested by the t-test.

The maximal observed increase in the serum iron concentration varied in the blood donors between 68 and 364 γ per cent, with an average increase of 206 γ per cent, while the increase in the normal controls varied between 76 and 169 γ per cent, with an average increase of 105 γ per cent. When tested by the t-test the maximal observed increase in serum iron concentration in these two groups showed a significant difference at the 5 per cent level.

Two of the normal controls thereafter started to donate blood and gave 500 ml blood (iron absorption tests included) at intervals of approximately 3 months. Before each donation hemoglobin and hematocrit determinations, red cell counts and iron absorption tests were performed, and the mean corpuscular hemoglobin content was calculated. The hemoglobin concentration, red cell count and mean corpuscular hemoglobin content remained practically constant during the experiments, but after a few donations the iron absorption curves showed marked alterations.

e curves of I. P. are shown in
2. On account of her iron ab-
ion test on June 7, 1957 we
lered it unjustifiable to allow
onate again without additional
She was therefore given iron
nent as described above, and
week after the conclusion of
nent another iron absorption
was performed. As this still
ed a high uptake of iron, a
d course of treatment was gi-
The subsequent test on August
957 indicated that the iron
s were still depleted, and
s decided that further treat-
was advisable. By mistake she was al-
to donate again at this time, but in
of this, after her third course of treat-
the iron absorption test on November
57 resulted in a flatter curve, similar to
riginal one of May 28, 1956, indicating
he had once more built up her reserves
n.

e curves of J. E. are shown in Fig. 3,
lemonstrate that the rise in serum iron
onsiderably greater after the second and
donation than in the first two tests.
ter the first absorption tests,
the blood donors were given
on treatment described above
the object of discovering
ner their absorption curves
l, as a result, take on a more
ned shape, indicating that the
depots were full.

e curves of H. H. are shown
g. 4. The first curve illustrates
st on May 23, 1956. After a

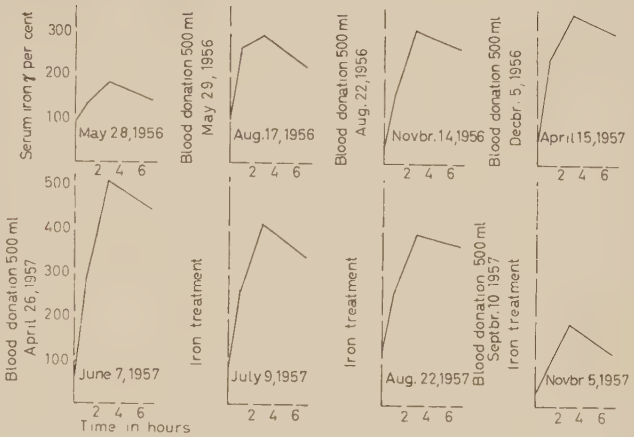

Fig. 2. Iron absorption tests on blood donor I. P. before her first donation and after blood donations and iron treatment.

course of iron treatment a second test was performed without a significant alteration in the shape of the curve. As this subject had previously donated blood on about 35 occasions, a further course of iron treatment was given on the supposition that the dosage had been insufficient. Following this, on August 27, 1956, the iron absorption test showed a greatly flattened curve. A third course of iron treatment was then given, and the subsequent test on October 12, 1956 resulted in a similarly shaped curve. This subject now began

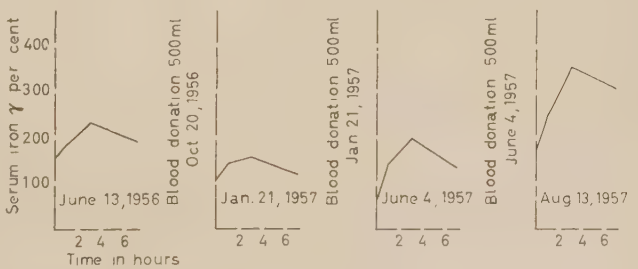

Fig. 3. Iron absorption tests on blood donor J. E. before his first donation and after blood donations.

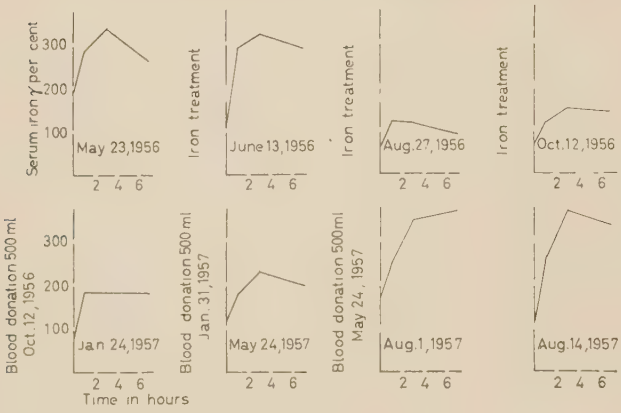

Fig. 4. Iron absorption tests on blood donor H. H. For further explanation see Table I and text.

again to give blood at 3-monthly intervals, with an iron absorption test before each donation. It was remarkable that, although it must be assumed that the iron depots were full on October 12, 1956, after only one donation the iron absorption test showed an increased uptake, and this became more marked with each further donation. In order to ascertain that the changes in the curves were reproduceable and had not occurred by chance, a further test was performed on August 14, 1957, after a 13 days interval in which there was no further blood donation or iron treatment. The results was strikingly simila to that of August 1, 1957.

Another noteworthy observatio in this subject is that as the iro depots were filled, the fasting seru iron concentration decreased, an only began to rise again when th resumption of blood donations onc more depleted the iron depots.

Figs. 5 and 6 also show absorp tion curves from blood donors wh were re-examined after iron treat ment. Both had a lower fastin serum iron after treatment tha before, while the absorption curve clearly show the change in shape whic characterises filling of the depots.

CONCLUSIONS

The following conclusions can be draw from this investigation:

1. Blood donors should be given additiona iron from the very first donation.
2. Determination of the fasting serum iro concentration appears to be an unreli able test of the state of the individual iron reserves. This, however, is not sur prising, since the test is merely a meas

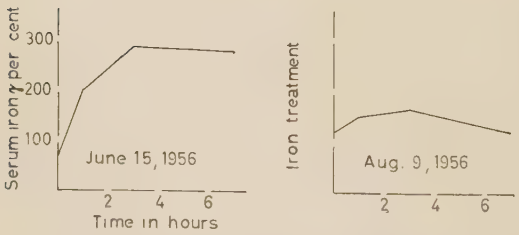

Fig. 5. Iron absorption tests on blood donor L. G. For additional data see Table I.

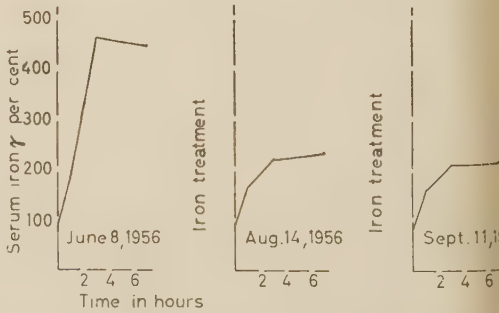

Fig. 6. Iron absorption tests on blood donor B.W. For additional data see Table I.

urement of the amount of iron in transit within the body.

The iron absorption test appears to give a reliable indication of the degree of filling of the iron depots.

SUMMARY

ron deficiency and the effect of iron tment are studied in blood donors by the absorption test. It is concluded that d donors should be given additional iron the very first donation, and that the rmination of the fasting serum iron conration appears to be an unreliable test he state of an individual's iron depots, reas the iron absorption test appears to a reliable indication of the degree of ng of the iron depots.

REFERENCES

man, J. C.: Treatment of chronic blood donor anemia. Ann. Int. Med. *34,* 393, 1951.

gmann, H. & Vogl, W.: Blutspenderkapazität und Verhütung von Spenderschäden. Wien. klin. Wchnschr. *68,* 135, 1956.

chner-Mortensen, K. & Olsen, C.: Colorimetric method for the determination of iron in serum. Compt. rend. Lab. Carlsberg, serie chimique *23,* 235, 1940.

leck, H. & Remy, D.: Orale Eisenbehandlung mit Beobachtung der Eisenresorption und der Restpolyglobulie. Ärtzl. Wchnschr. *7,* 316, 1952.

— Eisenmangel bei Polycythämie. Klin. Wchnschr. *31,* 155, 1953.

meyer, L. & Begemann, H.: Handbuch d. inn. Med. Bd. 2, 2. Auflage. Springer Verlag, Berlin-Heidelberg, 1951, p. 175.

länder, L. & Stampfli, K.: Der Schutz des Spenders im Blut-Spendedienst des Schweizerischen Roten Kreuzes. Bibl. Haemat. *5,* 143, 1956.

Jasinski, B.: Die Bedeutung der Eisenresorptionsversuche für die Diagnose und Differentialdiagnose der Eisenmangelanämien, insbesondere für die Erkennung der Eisenmangelzustände ohne Anämie. Schweiz. med. Wchnschr. *79,* 1255, 1949.

— & Roth, O.: Larvierte Eisenmangelkrankheit. Benno Schwabe Co., Basel 1954, pp. 47, 118.

Kölwel, E.: Kann Blutspenden schädlich sein? Münch. med. Wchnschr. *94,* 2479, 1952.

Matthes, M. & Kramer, G.: Der Eisenbedarf des Blutspenders. Dtsch. med. Wchnschr. *81,* 1262, 1956.

Möller, H.: Untersuchungen am Blutspender, insbesondere Serumeisen und Serumeiweiss-Bestimmungen. Ztschr. ges. inn. Med. *7,* 341, 1952.

— & Jürgens, J.: Untersuchungen am Blutspender; Veränderungen des Blutbildes und der Blutgerinnung. Zschr. ges. inn. Med. *7,* 1120, 1952.

Moore, C. V. & Dubach, R.: Metabolism and requirements of iron in the human. J. A. M. A. *162,* 197, 1956.

Remy, D., Goldeck, H. & Krüger, E.: Eisenmangel bei häufigem Blutspenden. Dtsch. med. Wchnschr. *77,* 1253, 1952.

— & Zöckler, H.: Medikamentöse Eisenprophylaxe bei Dauer-Blutspendern. Blut *2,* 32, 1956.

Roth, O.: Eisenmangelerkrankungen bei Nicht-Anämischen. Schweiz. med. Wchnschr. *80,* 1065, 1950.

Santy, A. C.: The response of blood donors to iron. Am. J. Med. Sci. *201,* 790, 1941.

Sturgis, C. C.: Hematology. Charles C. Thomas. Springfield, Ill. 1955, p. 66.

Triangle: From our laboratories: Iron deficiency in blood donors. Triangle *3,* 31, 1957.

Wachsmuth, W., Lutzeyer, W. & Heinrich, G.: Die Gefährdung des Spenders als aktuelles Problem des Blutspendewesens. Ärtzl. Wchnschr. *8,* 1209, 1953.

Whitby, L. E. H. & Britton, C. J. C.: Disorders of the blood. Churchill, London 1953, p. 154.

Scandinav. J. Clin. & Lab. Investigation 108—109, 10, 1958.

A SIMPLE CLINICAL METHOD FOR THE DETERMINATION OF SALAZOPYRIN (AZULFIDINE) IN SERUM

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(Received for publication October 16, 1957)

During clinical trials with new compounds of sulfa drugs of the type Salazopyrin (salicyl-azo-sulfa-pyridine, NNR Azulfidine) for the treatment of ulcerative colitis, there has been need of a simple method for assay of salazopyrin in serum. Earlier, von Porat (1944) has published two methods. One of these, however, has a high blank value and can not be used in the presence of jaundice or hemolysis, the other is rather elaborate and time-consuming.

The methods are based on the strong orange color of salazopyrin in alkaline solution. The difficulty has been to eliminate or compensate the color of serum itself. It has now been found that the simplest way is to acidify one portion of the serum, following which the salazopyrin is decolored and the acid serum sample may be used as a blank in colorimetric measurements. A prerequisite is, of course, that serum has the same optical density at alkaline and acid reaction. This is demonstrated by the finding of an average difference of 0 01 between the optical densities in alkaline (pH > 11) and acid (pH = 1.3) solution for 30 serum samples, selected at random. This difference is not statistically significant. A difference of the same order of magnitude is found with sera that show moderate hemolysis, as well as with icteric sera with icterus index (Meulengracht) up to 1:60 (Table I).

Salazopyrin in alkaline solution has its maximal optical density at 455 mμ. The difference in optical density between alkaline and acid solution also has its maximum at this wavelength (Fig. 1). The optical density of a serum solution of 1 mg per cent salazopyrin at alkaline reaction (Beckman B, 1 cm cuvette) at 455 mμ has been found to be 0.57.

Table I. *Optical density of icteric sera at alkaline and acid reaction.*

Icterus index	$E_{alk.}$	E_{acid}	$E_{diff.}$
1:10	0.38	0.41	—0.03
1:12	0.34	0.35	—0.01
1:24	0.47	0.49	—0.02
1:33	0.76	0.75	+0.01
1:60	1.47	1.50	—0.03
		Average difference	0.02

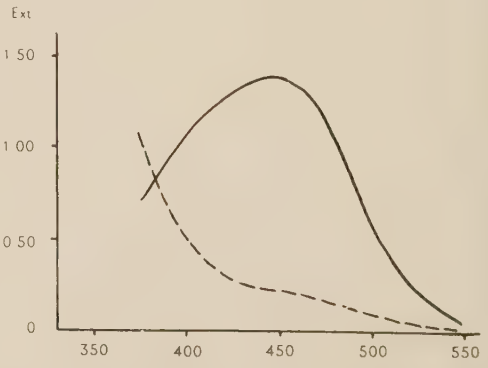

Fig. 1. Absorption curves of 10 mg per cent salazopyrin at pH > 11 (——) and pH = 1.3 (— — —).

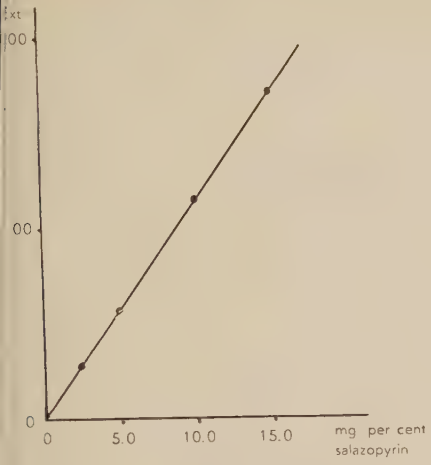

Fig. 2.

METHOD

) ml serum is diluted with 4.0 ml 0.1 N NaOH the optical density is determined at 455 mμ Beckman B photometer (1 cm cuvette) against ank of 1.0 ml serum diluted with 4.0 ml N HCl.

CALCULATION

$\times \frac{1}{0.57} \times 5 = E \times 8.80$ mg per cent salazo-

That different concentrations of salazopyrin give a straight line of optical density is shown in Fig. 2.

When 10 mg per cent salazopyrin were added to 20 different serum samples, the recovery was 10.0 ± 0.47 mg per cent. A recovery experiment is shown in Table II.

The method also has been used for another similar compound, salazodimidin, which is salicyl-azo-sulfa-dimethylpyrimidine. The calculation factor in this case is 9.25.

Table II. *Recovery experiment.*

Added amounts of salazopyrin	Recovered amounts of salazopyrin	Recovery in per cent
1.0	0.88	88
2.0	1.93	97
5.0	4.93	99
10.0	9.77	98

SUMMARY

A simple assay method for salazopyrin and analogous compounds in serum is described.

REFERENCE

Porat, B., von: Salazopyrin assay in serum, two methods. Nord. Med. *24,* 2070, 1944.

Scandinav. J. Clin. & Lab. Investigation 110, 10, 1958.

A SIMPLE METHOD OF CLEANING MERCURY

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(Received for publication August 1, 1957)

The methods of cleaning mercury recommended in textbooks (*e. g.* Peters & van Slyke, 1932; Harrison, 1947) are relatively laborious requiring cleaning towers of about one meter's height. During our work we have found a more simple method which we think may interest others and which is briefly described below. The method is capable of cleaning grossly contaminated amalgam-containing mercury leaving adherent films on glass.

The principle of the method is dripping of the mercury into dichromate cleaning fluid consisting of 500 g sodium dichromate, 5 litres concentrated sulphuric acid, and 5 litres distilled water, a volume of this fluid in ml corresponding to the weight of the mercury in g being adequate.

It is important that the mercury drips through the cleaning fluid in fine drops, and this can be achieved by means of a glass funnel connected to a fine glass jet with a short piece of rubber pressure tubing. The cleaning fluid is placed in a glass cylinder in such a way that the distance from bottom to surface is about 20 cm, and the mercury is allowed to drip through the fluid and collect on the bottom of the cylinder where it is left overnight. Subsequently the mercury is thoroughly washed with water untill the washings are neutral and dried by means of filter paper.

As a rule one passage through the cleaning fluid was sufficient to clean the mercury perfectly, but occasionally two passages were required. The weight loss of a very dirty sample of mercury weighing 958 g passed twice through cleaning fluid in this way was found to be 7.5 per cent.

REFERENCES

Harrison, G. A., Chemical Methods in Clinical Medicine. Third Edition, J. & A. Churchill, London 1947, p. 564.

Peters, J. P. & van Slyke, D. D. Quantitative Clinical Chemistry, Volume II, Williams & Wilkins, Baltimore, 1932, p. 45.

dinav. J. Clin. & Lab. Investigation 111—112, 10, 1958.

A CAPILLARY INK PEN FOR WRITING ON KYMOGRAPH PAPER

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(Received for publication November 9, 1957)

he classical method of recording in the siology laboratory is a smoked paper m. For certain purposes it is convenient ecord on white paper with an ink record- device.

he author has for many years used a of his own design. This pen and the ious steps in making it are shown in . 1. One of its basic parts consists of a ethylene tubing with an external dia- er of 2.4 mm and an internal diameter .9 mm. A piece of tubing, about 120 mm , is bent at right angles (Fig 1 a) over iniature gas flame. Each leg is about 42 when the pen is 35 mm long. The bend- is easier and the angle is more exact if ore of tin is inserted into each end of tube. The commercial tin for radio work e minor size) fits well into a 1.9 mm hole. er bending, the tube is allowed to cool about 10 minutes before the tin cores are ndrawn. The tube is then cut to size ac- ding to Fig. 1 b. A capillary of stainless l with a brass fitting is made from a ular injection needle No. 16 (0.60 mm rnal diameter) by shortening the steel illary to 3 mm (Fig. 1 c). The original ss fitting is turned on a lathe and cut n to an external diameter of 2 mm and ngth of 4 mm. The tip of the needle is fully polished with emery polishing paper a hard Arkansas stone. This reshaped dle is inserted into the bent tube to give complete pen shown in Fig. 1 d.

is advisable to use a type of ink which

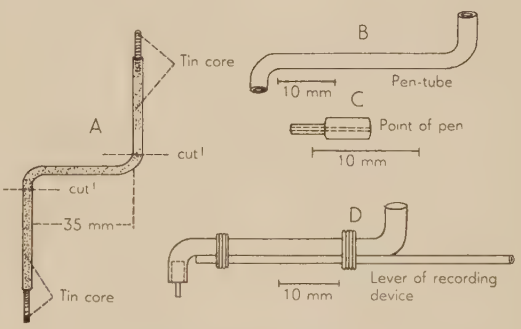

Fig. 1. Capillary ink-pen in different stages. A. Polyethylene tube with tin core and bent at right angles. B. The pen-tube cut to size. C. Point of pen. D. Pen mounted on lever.

does not easily clog the pen. The following material has proved satisfactory: Fountain pen ink, 12 parts by volume to which is added 1 part by volume glycerol. Red and green types are preferable as well as certain light blue ones. Other tend to clog. The pen is filled from the free opening of the tube by the aid of a small greased syringe. Rinsing, which rarely is necessary if a suitable ink mixture is used, can be affected by pressure from the syringe and by using a rinsing needle. It is advantageous to reshape the free end of the pen tube with a warmed conical metal rod which is pressed into the lumen so as to give the free tube end an internal conical shape that fits the external cone of the syringe. To obtain less inertia, a pen of considerably lower weight can be made on the same principle. This is achieved by using

a thinner tube with an external diameter of 1.8 mm, a point of stainless steel No. 18 (0.47 mm external diameter) which is cut to 1.5 mm and a brass fitting 1.4 × 2.5 mm. A complete pen with a tube 60 mm long has a weight of only about 250 mg including the ink.

It should be noted that frequencies above 10 cy/sec can not be recorded reliably with this type of ink pen. When used on electro-magnetic signals and time markers, the stroke should be small and excessive power (*i. e.* strong current) avoided. Otherwise the ink may flow from the pen through inertia. The pen point should be perpendicular to the surface of the paper and the pressure as low as possible. The pen is mounted on the lever of any standard recording device with glue and thread.

dinav. J. Clin. & Lab. Investigation 113—114, 10, 1958.

A SIMPLE CALF AND FOOT PLETHYSMOGRAPH

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(Received for publication November 22, 1957)

n open plethysmograph, originally in-ed for the study of orthostatic patients, constructed by the authors in 1954 at rehabilitation centre of Gothenburg. e the apparatus has proved useful in the nination of "psychoneurotic" cases with tative disturbances, several psychiatric cs have expressed their interest in the hod, and a brief description may be ified.

ig. 1 shows the sheet iron container A plast knobs for the lid; plast floor B; -piece C (plast bottom, brass plate); two-e plast lid D, in the figure turned upside n to show glass tube under the floater ; wooden knee-bridge E; one of the two s clamp-screws F, fixed to the brim of container to keep knee-bridge from ng; wooden recorder bridge G with brass , forked at the end and pierced by the spindle of the recorder lever. The floater ade from a 60 mm light metal lid with andard (cedar wood, 2 × 4 × 150 mm) d to it. A needle through the bottom es in a narrow glass tube. The top of standard is forked, the points of the fork ying tiny notches for the free recorder dle. The recorder lever (cedar wood, 2 × 175 mm) hinges by its horizontal spindle from the staff of the recorder ge, while its horizontal free spindle hin needle through the lever) rests upon top points of the floater. The distance een the spindles, as well as between the and the standard in position, is 11 mm. the free end of the lever are wedged a steadying wood splint and a small paper slip carrying the writing glass needle. It is essential that floater and recorder should have little mass and low vibration tendency.

A water temperature of 36° C falls only slightly, by about 2°, during an hour, corresponding to a decrease of the water volume of 11 ml, that is, .0002 per degree.

A rubber air cuff round the distal part of the thigh permits application of different pressures on the thigh vessels. (Air cuff round the ankle for shutting off foot circulation has been used in some experiments, too.)

At the beginning of the examination, the strictly vertical movement of the writer is tested and calibration made by repeated addition of 10 ml of water. After the examination, leg volume is determined by adding known quantities of water until the lever reaches its former position again.

The apparatus permits study of local systolic pressure, local venous pressure, respiration waves, pulse waves, tissue fluid accu-

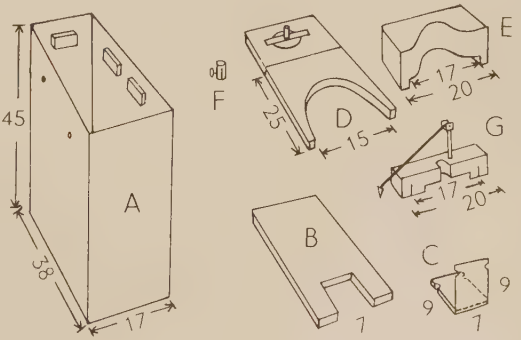

Fig. 1.

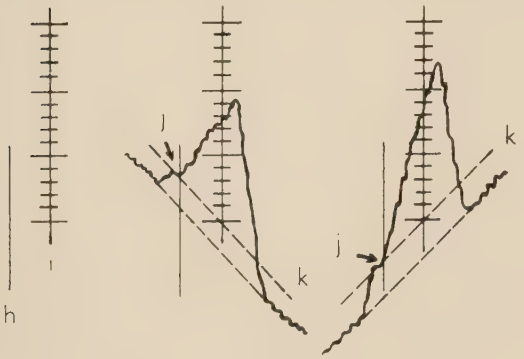

Fig. 2.

mulation and blood flow, as well as orthostatic, pharmacological, sensory and psychological reactions.

For measurement of blood flow *ad modum* Brodie (1905), see Barcroft (1953). In examinations where the stasis curve rises from an oblique basic curve, a modification is called for, as in Fig. 2. A vertical line *h* and a millimeter scale *i* are etched upon a transparent plate. From a point *j* on the stasis curve a line *j*—*k* is drawn, parallel to the interrupted basic curve. The plate is placed with *h* going vertically through *j* and the distance between stasis curve and the line *j*—*k* is determined from the millimeter scale. This value depicts the blood flow during the time elapsed when the kymograph paper has moved from *h* to *i*. Barcroft's formula (l. c., p. 149—150) then gives the real value of the blood flow.

REFERENCES

Barcroft, H. & Swan, H. J. C.: Sympathetic control of human blood vessels. Edw. Arnold & Co., London 1953, p. 141—151.

Brodie, T. G. & Russel, E.: On the determination of the ratio of blood flow through an organ. Proc. Physiol. Soc., J. Physiol. *32,* XLVII, 1905.

Letters to the Editors

linav. J. Clin. & Lab. Investigation 115, 10, 1958.

THE HYPERBILIRUBINEMIC AND HYPERFERRICEMIC ACTION OF NICOTINIC ACID

ita presented by Mattei (1946), Franchi isolo (1949), Belsasso, Risolo & Zuc- (1951) and Laurell (1953) suggest the ibility that nicotinic acid increases the avascular hemoglobin break-down. Dur- he first hour after an intravenous injec- of nicotinic acid, the serum bilirubin rally increases at the same rate as the n iron. Laurell (1953) assumed the oglobin break-down during this time to bout 4 times normal. It is, however, not vn how nicotinic acid brings about this k-down.

hen non-viable but compatible red cells given to healthy subjects, a similar in- se in serum iron and serum bilirubin is uced. 50 ml of blood stored in citrate, per cent) for 14 days at 4° C was admi- red within 5 minutes. The serum iron serum bilirubin were determined before, inutes and 60 minutes after the infusion at the same interval after the injection cotinic acid. Two examples of the results ined are given in the table.

ccording to investigations of Ross, h, Peacock & Sammons (1947) it can oncluded that some 25 per cent of red stored in citrate for 14 days disappear the circulation within 1 hour. Bothwell, tado, Donohue & Finch (1956) found when the blood had been stored for 21 , some 50 per cent of the red cells dis- ar within 2 hours of infusion.

ne observation set forth above may sug- that the injection of nicotinic acid leads estruction mainly of aged erythrocytes.

Table I. *Serum bilirubin and serum iron concentrations after infusion of stored blood* (A. — subject: P. B. 25 years) *and after intravenous injection of nicotinic acid* (B. — subject: G. F. 28 years).

Minutes after injection	Serum bilirubin mg/100 ml		Serum iron µg/100 ml	
	A	B	A	B
0	0.93	0.62	163	118
30	1.08	0.70	168	127
60	1.68	1.23	236	182

REFERENCES

Belsasso, E., Risolo, A. & Zucconi, V.: Comportamento della sideremia nelle iperbilirubinemie provocate da acido nicotinico. Minerva Med. *2*, 518, 1951.

Bothwell, T. H. Hurtado, A. V., Donohue, D. M. & Finch, C. A.: Erythrokinetics IV. The plasma iron turnover as a measure of erythropoiesis. Blood *12*, 409, 1957.

Franchi, G., & Risolo, A.: Ricersche sulle bilirubinemie provocate e sull'escrezione dell'ossisantonina in rapporto alla patologia epatica. Arch. Sci. Med. *88*, 55, 1949.

Laurell, C.-B.: Influence of nicotinic acid on the intermediary metabolism of iron and bilirubin. Acta pharmacol. toxicol. *9*, 86, 1953.

Mattei, C.: Sui vari aspetti della curva bilirubinemica da carico di acido nicotinico nei normali e negli epatopazienti. Minerva Med. *37*, 308, 1946.

Ross, J. P., Finch,, C. A., Peacock, W. C. & Sammons, M. E.: The in vitro preservation and post-transfusion survival of stored blood. J. Clin. Investigation *26*, 687, 1947.

Malmø General Hospital,
Malmø, Sweden.
November, 1957. *K. Gydell.*

Scandinav. J. Clin. & Lab. Investigation 116—117, 10, 1958.

EFFECT OF ETHER-INSOLUBLE PHOSPHATIDES ON PLASMA RECALCIFICATION TIMES

In experiments on dairy produce ether insoluble phosphatides were found which accelerated the recalcification time of human plasma. For comparison the ether-insoluble phosphatides were extracted from ox-brain and their effect on recalcification times observed.

METHOD

Macerated ox-brain, after dehydration and extraction with acetone and ether, was extracted with hot ethanol. The ethanol extract was evaporated to dryness and the material obtained was called the crude ether-insoluble fraction. This was treated with pyridine according to Rosenheim & Tebb (1910) thus separating the previous fraction into cold pyridine-soluble and insoluble parts, the former containing the crude cerebrosides, the latter the crude sphyngomyelins.

The sphyngomyelins were purified by the method of Thannhauser, Benotti & Boncoddo (1946). The insoluble material was dissolved in chloroform, precipitated by five volumes of acetone, treated with 0.25 N NaOH for two days at 4° C, acidified, washed and the precipitate suspended in warm 9:1 petroleum-ether-methanol mixture. The insoluble brown residue was discarded, the supernatant solution cooled to 4° C and the white precipitate purified by repeated solution in warm n-propanol.

The cerebroside fraction was purified by acetone precipitation from pyridine.

Both fractions were refluxed with diethyl-ether for three hours, the insoluble material centrifuged out, dissolved in warm ethanol and crystallized by cooling to 0° C. The cerebroside crystals appeared like clusters of flat oyster shells. The sphyngomyelin crystals were long, thin needles, soluble in warm glacial acetic acid, and benzene, but insoluble in cold ethanol; gave negative Blix, Molisch and Schiffe tests; and dispersed to give emulsions in water.

The cerebroside was soluble in cold ethanol and pyridine, gave negative Schiffe and Blix, but a positive Molisch test.

The materials were stored under n-propanol and before use were washed with acetone and ether to remove all the propanol. The resulting powders were finally dissolved in veronal buffer to give concentrations of 50 mg/100 ml.

The recalcifications were performed as previously described (Kingsbury & Morgan, 1957) using 0.1 ml of phospholipid solution and 1 ml of fresh human fasting plasma.

RESULTS

These are from three separate extractions, using one test plasma for each and are the averages of three consistent series for the substances of one extraction (see Table). The figures for the controls are the averages of all control series performed with the same plasma before, between, and after the test substances from one extraction.

DISCUSSION

These results show that, although the crude ether-insoluble residues were inactive, a coagulating action appeared in the sphyngomyelin fraction following pyridine extraction which increased as the material was purified. Also, an anticoagulant, apparently as powerful as the sphyngomyelin coagulant, appeared in the cerebroside fraction. This activity, however, disappeared on purification. The cerebroside crystals, thought to be mostly phrenosine, had no effect on the recalcification times.

SUMMARY

From the crude ether-insoluble phospholipids of ox brain were separated a coagulant

Table I. *Effect of extractions on recalcification time.*

t material	Clotting times of extractions					
	1		2		3	
	min.	sec.	min.	sec.	min.	sec.
trols	5	30	5	55	4	43
de ether-insoluble	6	8	5	58	5	7
d pyridine-insoluble	4	15	4	52	3	34
d pyridine-soluble	8	53	9	14	7	48
d pyridine insoluble from above (Sphyngomyelins)						
owing one hydrolysis	4	10	4	53	3	47
lowing two hydrolysis	3	35	4	26	3	16
r precipitation from pet. ether-methanol	3	40	4	15	3	15
precipitation from hot propanol	3	52	4	15	3	20
precipitation from hot propanol	3	33	3	51	3	5
luxed with ether	3	15	3	47	3	5
rystalisation from hot ethanol	3	4	3	22	2	54
d pyridine-soluble from above (Cerebrosides)						
er 2nd pyridine extraction	8	32	8	57	7	13
luxed with ether	6	15	6	20	5	42
rystalisation from hot ethanol	5	22	5	34	5	00

ich could be ascribed to sphyngomyelin; nactive cerebroside; and a powerful anti-gulant which was soluble in cold ethanol cold glacial acid, and insoluble in ether. periments are in progress to define this icoagulant.

rgical Unit,
Mary's Hospital,
ndon, England.

August, 1957.

REFERENCES

Kingsbury, K. J., & Morgan, D. M.: The effect of the lipids from human chylomicrons on the recalcification times of the plasma. Clin. Sci. *16,* 589, 1957.

Rosenheim, O., & Tebb, M. C.: The lipoids of the brain, Part II. A new method for the preparation of the galactosides and of sphyngomyelin. J. Physiol. *41,* Proc. i—ii, 1910.

Thannhauser, S. J., Benotti, J. & Boncoddo, N. F.: The preparation of pure sphyngomyelin from beef lung and the identification of its component fatty acids. J. Biol. Chem. *166,* 677, 1946.

K. J. Kingsbury.
D. M. Morgan.

GLUTAMIC OXALACETIC TRANSAMINASE AND ZONE ELECTROPHORESIS OF SERUM

The determination of serum glutamic oxalacetic transaminase (SGOT) has shown to be of diagnostic value in various diseases associated with tissue necroses. Among the investigations of physicochemical properties of this enzyme no reports concerning the electrophoretic mobility have as yet been available.

The preparative zone electrophoresis of serum as described by Nikkilä, Haahti & Pesola (1953) seemed to us to be a convenient method to determine the localisation of SGOT in relation to serum electrophoretic protein fractions. This was carried out in polyvinylchloride — Geon 426 — resin using 0.05-M medinal-HCl buffer pH 8.6. After running 36 hours the resin was cut into ½-cm strips. From each of these the proteins were eluated with 3.0 ml of saline solution. The protein content of each eluate was determined with the Folin-Ciocalteau-reagent as suggested by Kunkel & Tiselius (1951) using 0.1 ml of eluate. The SGOT activity of each eluate was determined by the method of Umbreit, Rahway, Kingsley, Schaffert & Siplet (1957). Considering the low GOT activity of normal sera as determined by this method and the incomplete protein recovery with saline elution, a serum with elevated GOT activity (200 units) was selected (myocardial infarction).

The localisation of SGOT activity in relation to protein distribution is demonstrated in Fig. 1. SGOT fraction lies between α_2 and β globulins as a rather narrow and distinct peak with maximum activity of 52 units. It has been stated in other connection that the Geon-426-resin is an indifferent medium with poor adsorptive properties on proteins. Thus it might be assumed that the result of

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February, 1958.

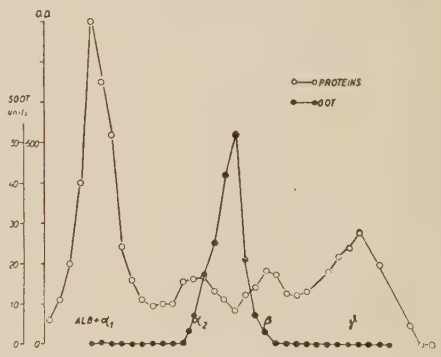

Fig. 1. Comparison of activity of glutamic oxalacetic transaminase with protein distribution in different zones of electrophoretic fractions of serum in a patient with myocardial infarction.

the present study demonstrates the electrophoretic mobility of SGOT without any specific disturbing adsorptive effects of medium.

The electrophoretic mobility of GOT in various tissues and pathologic conditions are under further study in our laboratory.

ADDENDUM

Since this paper was sent to publication a report of similar investigation has been abstracted in Transact. Internat. Congress of Clin. Chem. 1957 (Scand. J. Clin. & Lab. Invest. 10: suppl. 31) by G. Junger. Since no detailed data from the work has been available, the difference of results from our study is difficult to interpret.

REFERENCES

Kunkel, H. G. & Tiselius, A.: Electrophoresis of proteins on filterpaper. J. Gen. Physiol. *35,* 89, 1951.

Nikkilä, E., Haahti, H. & Pesola, R.: On the technique of preparative electrophoresis of proteins in supporting medium. Acta Chem. Scandinav. *7,* 1222, 1953.

Umbreit, W. W., Rahway, N. J., Kingsley, G. R., Schaffert, R. R. & Siplet, H.: A colorimetric method for transaminase in serum or plasma. J. Lab. & Clin. Med. *50,* 454, 1957.

E. Haahti,
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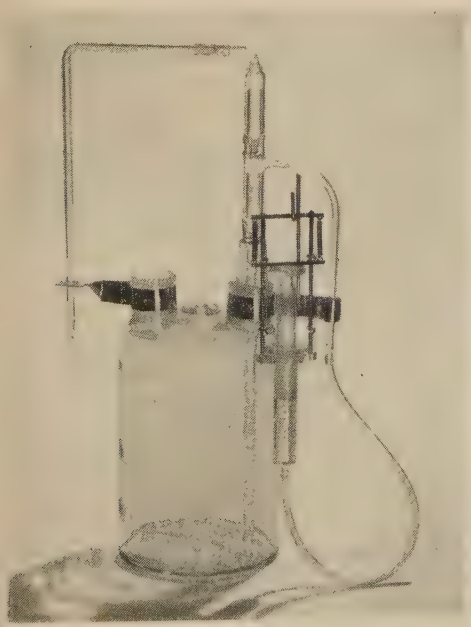

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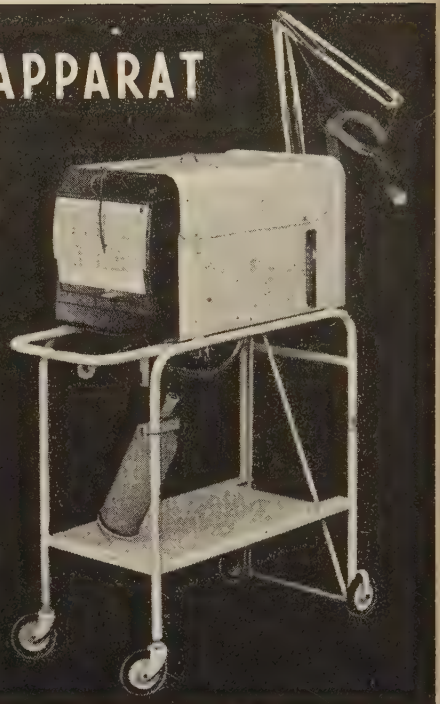

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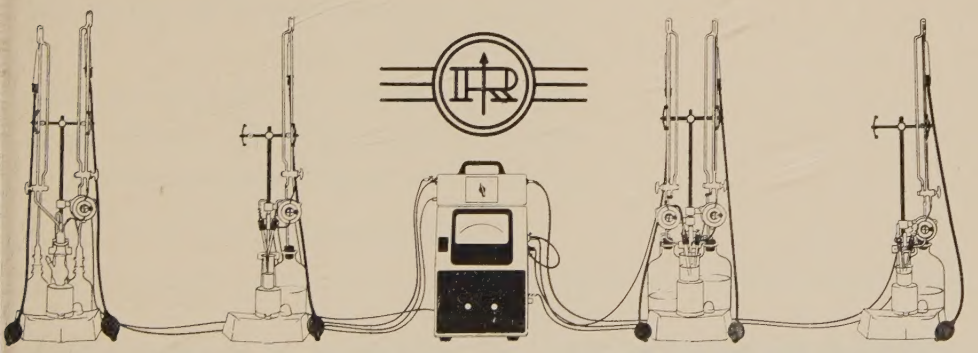

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DISTRIBUTED BY EJNAR MUNKSGAARD, COPENHAGEN, DENMARK

PRINTING ARRANGEMENTS BY OSLO UNIVERSITY PRESS, OSLO, NORWAY

PRINTED BY A. W. BRØGGERS BOKTRYKKERI A/S, OSLO